HSV2 INHIBITION WITH HIV SUPPRESSING NANOPARTICLES FOR TARGETED DRUG DELIVERY OF MACROPHAGES/MONOCYTES

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Submitted by
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Certificate

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LIST OF ABBREVIATION

VCV	–	Valacyclovir
NP	–	Nanoparticles
MMA-SPM	–	Methyl Methacrylate Sulfopropyl methacrylate
MPS	–	Mononuclear phagocyte system
PLGA	–	Poly (Lactic-Co-glycolide)
MMA	–	Methyl methacrylate
SPM	–	Sulfopropyl methacrylate
PEG	–	Poly (ethylene glycol)
PLA	–	Poly lactic acid
PCL	–	Poly Caprolactone
PLBA	–	Poly (β- benzyl L-aspartate)
POE	–	Poly ortho- ester
PA	–	Poly anhydrides
SA	–	Sebacic acid
CPP	–	P(Carboxyphenoxy) propane
CPH	–	P(Carboxyphenoxy) hexane
PHCA	–	Poly hexylcyanoacrylate
HAS	–	Human serum albumin
TNF	–	Tumor necrosis factor
KB	–	Kappa B

List of Abbreviation

HSV	–	Herpes simplex virus
HIV	–	Human immunodeficiency virus
VZV	–	Varicella zoster virus
TK	–	Thymidine Kinase
PIBCA	–	Poly Isobutyl Cyanoacrylate
STI	–	Sexually transmitted infections

SCOPE AND PLAN OF WORK

Herpes simplex virus (HSV) is a human DNA virus with two species, HSV-1 and HSV-2, that causes a variety of disease manifestations, the most common being localized recurrent skin and mucous vesicular lesions, occurring after primary infection (e.g., acute Herpetic Gingivostomatitis). Genital HSV is a sexually transmitted disease and there is strong evidence that it increases the risk of other infections through genital contact, in particularly HIV infection. Varicella-zoster virus (VZV) is a member of the herpes virus family. Primary infection is varicella (chickenpox), and its secondary infection is zoster (shingles). HSV2 prevalence is increasing worldwide and HSV2 is the major cause of genital ulcer disease (GUD) in the developed world. In the developing world, the major public health importance of HSV2 lies in its potential role as a co-factor for HIV transmission. WHO says that the HSV2 prevalence varies widely, with generally higher rates in developing than in developed countries and in urban than in rural areas.

WHO estimated that the HSV Prevalence is higher in the USA (22% in adults) compared with Europe (generally less than 15%). However, substantially higher rates are seen in Sub-Saharan Africa and the Caribbean, with prevalence in adults of around 50% in many countries.

Overall, prevalence is higher in women compared with men, especially among the young and rates of up to 40% have been recorded among women aged 15-19 in Kisumu, Kenya. HSV2 reactivation and duration of recurrences are significantly increased in HIV infected individuals. The frequency and severity of recurrences increases as CD4 cell count decreases. There is evidence that inclusion of Valacyclovir in antiretroviral therapy prolong survival in HIV seropositive individuals. One study has shown that HSV2 reactivation is associated with increases in plasma HIV1 RNA and intracellular gag mRNA and that plasma HIV1 RNA level decreases significantly during treatment with Valacyclovir. As a result, it is possible that HIV progresses more rapidly in untreated HSV2 positive individuals. However, evidence is inconclusive and more studies of the effect of episodic HSV2 therapy on HIV are needed, especially in developing countries**.** Valacyclovir to be included for treatment of severe herpetic ulcers especially HIV infected individuals. Valacyclovir is an antiviral drug, which has been the standard treatment for genital herpes, varicella-zoster virus (VZV) for the past decade in developed countries. For primary infection in children, i.e., herpetic gingivostomatitis, small recurrent therapies (RCTs) suggest that oral Valacyclovir reduces the mean duration of pain and other symptoms. A few RCTs document that the frequency of recurrent herpes labials can be reduced by antiviral

therapy. Valacyclovir appears to be as effective as oral Acyclovir and somewhat better than Famcyclovir. Valacyclovir is available in generic forms, contrarily to other more recent antiherpetic drugs. In this context we planned to prepare Valacyclovir Nanoparticles formulation to increase the bioavailability of drug through targeting to the macrophage/monocyte systems of the body. Through this targeted drug delivery we can able to reduce the required drug dose for the therapy. This enables the reduction in the side effects and toxicity.

(i) To develop the nanoparticles formulation with antiviral drug Valacyclovir for enhanced macrophages and monocytes drug delivery and to have a controlled release effect.

(ii) To enhance the bioavailability of Valacyclovir.

INTRODUCTION

PARTICULATE CARRIERS

For almost 45 years researchers have attempted to use the properties of particulate colloidal systems for controlling the biological dispersion of drugs. Such carriers can be as small as 10nm in diameter and it may be monolithic or capsular. Types include lipid vesicles, protein microspheres, emulsion and natural or modified endogenous particles.

The perceived advantage of particulate colloidal carriers is that they can carry and protect an extremely high payload of drug while following their own biological pathways. However, particulate carriers are generally recognized by the body's immune system and captured by the cells of the MPS; also, they cannot easily escape from the circulation, except where the endothelia are either discontinuous or damaged.

Particulate colloidal carriers have been considered for both intravascular targets. Much recent attention has been paid to adjusting the surface of such materials in an attempt to fool the immune system into treating these drug carriers as being non-foreign. Such stealth approaches will increase the probability that the carriers will be able to interact with features of the target site, or will persist as circulating depots of drug in the central blood compartment.

Various factors affecting the release of drugs from particulate carriers are such as,

Drug

- Position in the particle
- Molecular weight
- Physicochemical properties
- Drug-carrier interaction
- Diffusion; desorption from surface

Particles

- Type and amount of matrix material
- Size and density of the particle
- Capsular or monolithic
- Extent and nature of any cross-linking; denaturation of polymerization
- Presence of adjuvants
- Surface erosion; particle diffusion and leaching
- Total disintegration of particles

Environment

- Hydrogen ion concentration
- Polarity
- Ionic strength
- Presence of enzymes
- Temperature
- Microwave
- Magnetism

- Light (Vijayakumar, Gilbert.S.B., 2001)

NANOPARTICLES

Controlled and targeted delivery is one of the most enviable requirements from a carrier, which involves multi-disciplinary site-specific or targeted approach. One attempt to achieve this goal was the development of colloidal drug carriers known as Nanoparticles. The concept of using nanoparticles as a vehicle for drug delivery was first developed by Speiser and co-workers in the late 1960s and early 1970s. Nanoparticles are solid colloidal particles ranging in size from 10 to 1000 nm (1micrometer). In which the active principle (drug or biologically active material) is dissolved, entrapped, and/or to which the active principle is adsorbed or attached. Nanoparticulate drug delivery system may offer plenty of advantages over conventional dosage forms, which include improved efficacy, reduced toxicity, enhanced biodistribution and improved patient compliance.

DRUG RELEASE

The drug release from nanoparticles can be occurred by different methods.

- Desorption of surface bound drug
- Diffusion through the nanoparticle matrix
- Diffusion through the polymer wall
- Nanoparticle matrix erosion

- A combined erosion diffusion process.

POLYMERS EMPLOYED AS NANOPARTICLES

Synthetic polymers:

- Poly methyl methacrylate
- Poly methyl methacrylate copolymers
- Poly methyl cyanoacrylate
- Poly isobutyl cyanoacrylate
- Poly hexyl cyanoacrylate
- Ethyl cellulose
- Eudragit RL
- Eudragit RS

Natural polymers:

- Serum albumin
- Gelatin
- Lecithin
- Collagen
- Casein

Types of Nanoparticles used as carrier for therapeutic and diagnostic agents

S. No.	Types of Nanoparticles	Materials used	Applications
1	Polymeric Nanoparticles	Biodegradable polymers	Controlled and targeted drug delivery
2	Solid lipid Nanoparticles	Melted lipid dispersion in an aqueous surfactant	Least toxic and more stable colloidal carrier systems as alternative materials to polymers
3	Nanosuspensions and Nanocrystals	Drug powder is dispersed in a surfactant solution.	Stable system for controlled delivery of water-insoluble drugs
4	Polymeric micelles	Amphiphilic block copolymer	Systemic and controlled delivery of poorly soluble drugs
5	Ceramic Nanoparticles	Silica, Alumina, Titania	Drug targeting, Bio-molecules delivery controlled and targeted drug delivery carriers for site specific drug delivery
6	Liposomes	Phospholipid vesicles	Controlled and targeted drug delivery
7	Dendrimers	Biodegradable polymers	Carriers for site specific drug delivery
8	Magnetic Nanoparticles	An inorganic core of iron oxide coated with polymer	Drug targeting , diagnostic tool in biology and medicine
9	Nanoshells coated with gold	Dielectric core and a metal shell.	Tumor targeting
10	Nanowires or Carbon Nanotubes	Metals, semiconductors or carbon Aerogel	Gene and DNA delivery
11	Nanopores	Aerogel	Controlled release drug carriers
12	Quantum dots	CdSe-CdS core-shell	Targeting, Imaging agents
13	Ferrofluids	Iron oxide magnetic nanoparticles surrounded by a polymeric layer	For capturing cells

Polymers used in nanoparticle formulations

Common classes of polymers used to encapsulate drugs in colloidal systems include polyamides, poly (amino acids), polyesters, poly-orthoesters and poly anhydrides.

Polyesters

Recently, biodegradable polyesters such as poly (lactic acid), poly (glycolic acid) and the copolymers of lactic and glycolic acid, i.e.,poly (lactide-co-glycolide). (PLGA) have been used extensively for biomedical applications. Being biodegradable they have the advantage of not requiring surgery for removal after they have served their purposes. They protect the entrapped drug against degradation and control its site specific delivery. They are synthesised through ring opening polymerisation of cyclic lactones. These copolymers (with varying lactide: glycolide ratios) are amorphous and easily dissolve in organic solvents such as dichloromethane and ethyl acetate. The degradation rate in water is a function of the molecular weight and the lactide:glycolide ratio. Higher glycolide content and lower molecular weight increase the degradation rate. As they are all strongly hydrophobic they are more efficient for encapsulation of hydrophobic drugs than hydrophilic drugs. A burst release is another characteristic observed with nanoparticles made from

PLGA. The drug release from PLGA microparticles often has a tri-phasic pattern *in vitro* as well as *in vivo*. A fast initial release phase (burst) followed by a second slow release phase lasting days or weeks and a third rapid release phase is seen in particular with peptide and protein drugs. The burst release is undesirable in most of the sustained-drug delivery applications, since the dosage of the potent drug encapsulated is required to be released over a long period of time. Even a small percentage of the drug immediately released would increase its local concentration to an extent near or above that at which the drug becomes toxic *in vivo*. The defence mechanism of the body would respond to this increased toxicity level by forming additional layers of tissues on the polymer surface, thereby disrupting the entire release profile and mechanism. Any drug released during the burst stage may be metabolized and excreted without being effectively utilized as it may not reach its target tissue. Except for vaccines, a tri-phasic drug release is generally not desirable for most drug therapies. Insufficient drug may be delivered to maintain the desired pharmacological effect in the slow release phase and toxicity problems may occur during the rapid initial and third release phases because of too high drug levels. The fast initial release (burst) is commonly attributed to drug localised on the surface of particles or to easily accessible drug, for example, in the case of highly porous microparticles. Thereafter, a

diffusion-controlled slower release phase follows. Finally, when the molecular weight of PLGA approaches a certain lower threshold, the weight of the microparticles decreases rapidly and an erosion-controlled rapid release phase occurs. Recently, the formation of a nonporous film around the microparticles after incubation in the release medium has been reported. The decreased surface porosity of the microparticles led to reduced drug permeability and resulted in the slow release phase without burst release.

Poly (Ethylene glycol) based block co polymers

The nature of the surface of the nanoparticles largely influences their biodistribution following *in vivo* administration. Hydrophobic nanoparticles are rapidly cleared from the systemic circulation by the MPS, ending in the liver or in the spleen. The hydrophobic nature of most biodegradable particles could limit the applicability of these carriers in many drug delivery applications. In order to overcome concerns of clearance by the MPS, surface modification techniques have been utilized. These modification techniques ultimately produce a particle that is not recognized by the MPS by rendering the surface of the particle hydrophilic. Several types of surface modified nanoparticles have been described in recent literature. The most common moiety used for surface modification is poly (ethylene glycol) (PEG). PEG is a hydrophilic, non-ionic polymer that

has been shown to exhibit excellent biocompatibility. PEG molecules can be added to the particles via covalent bonding or by surface adsorption. The presence of a PEG brush on the surface of nanoparticles besides increasing residence time in the systemic circulation can also reduce protein and enzyme adsorption on the surface and thus can retard particle degradation. The degree of protein adsorption can be minimized by altering the density and molecular weight of PEG on the surface. The stability of PLA particles has been shown to increase in simulated gastric fluid (SGF) with the addition of PEG on the particle surface. After 4 hours in Simulated Gastric Fluid, 9% of the PLA nanoparticles converted to lactate versus 3% conversion for PEG-PLA particles. PEG is also believed to facilitate transport through the Payer's patches of the Gut-associated lymphoreticular tissue. Usually PEG block co-polymers show quite different properties compared to the constituent polymers lactic acid and glycolic acid. PLGA – PEG block co-polymers are hence used as biomaterials with their own unique properties and classified as triblock, star block, branched or graft block co-polymers as illustrated in Fig. 1.

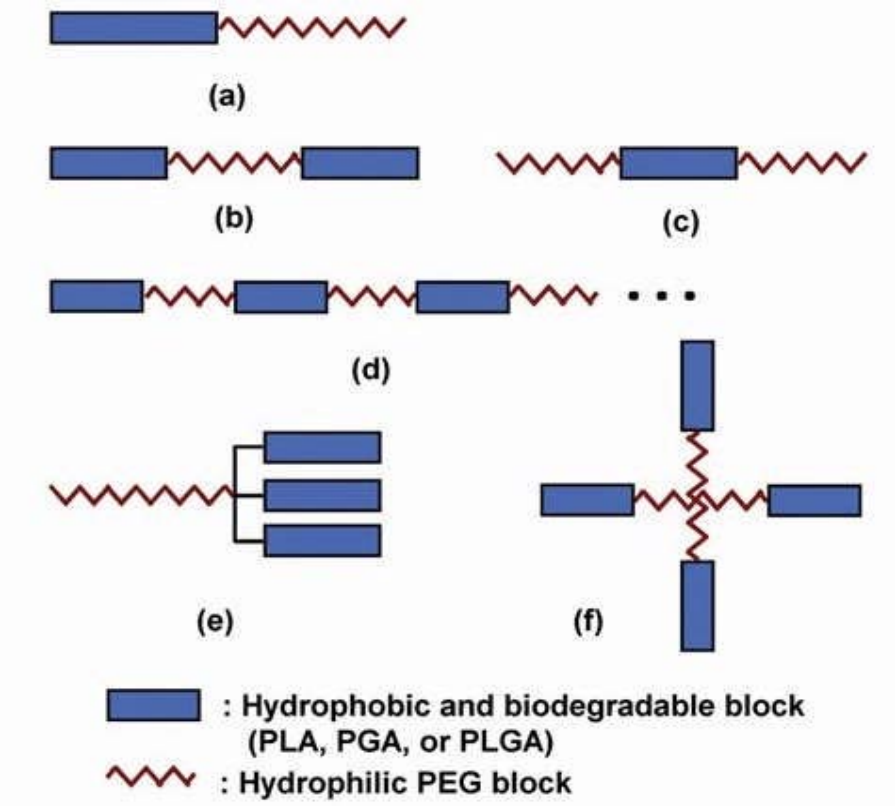


Fig. 1. Schematic representation of (a) Monblock (b) Diblock (c)Triblock (d) Multiblock (e) Multiblock branched or comb (f) Star shaped.

PLGA degradation shows autocatalysis wherein bulk degradation occurs with a decrease in pH resulting from the release of lactic acid and glycolic acid monomers. The resulting carboxyl groups, oligomeric PLGA can increase degradation of the PLGA formulation. Block co-polymers with PEG have also gained attention as an alternative for overcoming these side effects. Another main drawback of these polymers is their non specific interaction with cells and plasma proteins, leading to accumulation in

non target cells causing limitations in practical drug formulations. Hence, surface modified nanoparticles have been developed to control their interactions. PEG coated (sterically stabilised) nanoparticles can avoid sequestration by the Mononucleur Phagocyte system (MPS) and hence show increased circulation time in the body. However, these coated nanoparticles lack functional groups for ligand coupling. Some successful attempts have been reported for PEG systems where a terminal functional group has been added to PEG. Polysaccharide coatings have also been studied since they are alternative hydrophilic molecules. Some polysaccharides facilitate mucoadhesion or function as recognition factors allowing recognition. For example PEG Dextran or Dextran PLA. Biodegradable and biocompatible polymers such as poly (lactide) (PLA), poly (ε-caprolactone) (PCL), poly (β-benzyl L-aspartate) (PLBA), and poly (γ-benzyl L-glutamate) (PLBG) have been commonly used for the core material of micelles while PEO and PEG are used as hydrophilic blocks. Studies on polymeric micelles comprised of PEO as hydrophilic block and PCL, PLA, PLBA, PLBG as hydrophobic block have been carried out by many groups. Amphiphilic block copolymers have the ability to produce nanoparticles by self assembling in an aqueous environment. The hydrophobic blocks of the copolymer form the core of the micelle, while the hydrophilic blocks form the corona or outer shell. The solubility of the

hydrophobic drug in the aqueous media is greatly increased by the use of micelles. Thus, incorporating a drug in the micelle is an effective method of preparing an efficient drug delivery system.

Poly caprolactones (PCL)

Poly (ε-caprolactone) (PCL) obtained by ring opening polymerization of ε-caprolactone was first reported by Pitt *et al.*,(1990) for the controlled release of steroids and narcotic antagonists as well as to deliver ophthalmic agents. PCL, aliphatic polyester has been intensively investigated as a biomedical material. It demonstrates a low melting point (57°C) and a low glass-transition temperature (−62°C). Under physiological conditions, PCL can be degraded by microorganisms as well as by hydrolysis. Under certain circumstances, it is possible to enzymatically degrade cross linked PCL (termed enzymatic surface erosion). Low molecular-weight fragments of PCL are also reportedly absorbed by macrophages intracellularly. The rate of biodegradation for PCL is slower than other biodegradable materials thus making it suitable for design of long term implantable systems. For example, Capronor, a US FDA approved contraceptive device. Another interesting property of PCL is its propensity to form compatible blends with a wide variety of polymers. Molpeceres *et al.,(1999)* studied cyclosporine encapsulation within Polycaprolactone nanoparticles and reported 95% encapsulation efficiency

within the particles. They also showed that this formulation offered a good alternative to existing cyclosporine formulations as far as drug monitoring is concerned. Synthesis of amphiphilic copolymer based on Dextran grafted with PCL chains which significantly reduced protein absorption. Recently, Merle and coworkers reported encapsulation of vancomycin in biodegradable PCL microparticles for bone implantation.

Poly (alkylcyanoacrylates)

Poly (alkylcyanoacrylates) has been used as tissue adhesives in surgery since these are well tolerated in vivo. Unlike PLA and PGA, here only the side chains are biodegradable and not the backbone. Their delayed degradation characteristics thus do not generate an acidic environment during drug release. This has prompted intense research for their use in many nanoparticles formulations. The production of NPs by mechanically polymerizing the dispersed methyl or ethyl cyanoacrylate in aqueous acidic medium without irradation or an initiator in the presence of polysorbate-20 as a surfactant. Busulfan entrapment by nanoprecipitation into five different types of poly (alky cyanoacrylate) polymers. The polymers leading to the highest busulfan loading efficiencies were poly(isobutyl cyanoacrylate) (PIBCA) and poly (ethyl cyanoacrylate) nanoparticles displaying busulfan loading ratios equal to 5.9% (w/w) together with nanoparticle yields of 71% (w/w). The in vitro release studies

under sink conditions, in water, or in rat plasma showed a fast release in the first 10 min followed by a slower one over 6 h.

The potential of poly (alkylcyanoacrylate) (PACA) NPs to overcome multidrug resistance problems at cellular level and in relation to drug biodistribution. Resistant cells treated with doxorubicin loaded poly (alkylcyanoacrylate) nanoparticles showed a much higher sensitivity to the drug, relative to the free drug when compared with NPs using other biodegradable polymers. The mechanism proposed to explain the ability of doxorubicin-loaded PACA nanoparticles to overcome the resistance to doxorubicin in resistant cancer cells was based on the adhesion of the nanoparticles to the cell surface, followed by the simultaneous release of the drug and nanoparticles degradation product (poly cyanoacrylic acid) that combine as an ion-pair able to cross the cell membrane without being recognized by the Poly glycoprotein. To date, only the poly (alkyl cyanoacrylate) nanoparticles have been identified as to be fulfilling these requirements to overcome the resistance caused by the P- glycoprotein complex. Lipid nanocapsules prepared using poly (alkyl cyanoacrylates) were between 25 to 100 nm in size and showed fast initial release of 60 to 75% in the first 48 hours. However the major limitations associated with use of this polymer are the particle size, high cell uptake and toxicity.

Poly (ortho-esters) (POE)

Poly (ortho-esters) is another important group of hydrophobic polymer with drug delivery applications and are synthesised by the addition of polyols to diketene acetals. POEs possess acid sensitive ortho-ester linkages that undergo rapid hydrolysis at physiological pH and an even faster rate in an acidic pH. Therefore, incorporation of a small amount of acidic excipients may help to control the hydrolysis rate. On the other hand, incorporation of basic excipients stabilises the bulk of the matrix but facilitates erosion at the surface. The polymers conjugated with N- hydroxysuccimide were hydrolyzed in a biphasic mode, with a fast initial phase occurring in the first few hours, followed by a slower phase in the next few days. These ionomers represent a novel class of biomaterials with readily controllable physical and chemical attributes for tissue engineering.

Polyanhydrides (PA)

Polyanhydrides are hydrophobic and contain water sensitive linkages that may undergo hydrolytic bond cleavage to generate water-soluble degradation products. Surface erosion takes place due to water sensitive linkages. The majority of Polyanhydrides studied are based on Sebacic acid (SA), P- (carboxyphenoxy) propane (CPP) and

P-(carboxyphenoxy) hexane (CPH). The Sebacic acid component of biodegradable PAs is utilised as a surface eroding drug delivery device. A wide variety of drug and proteins have been incorporated into PAs and their modified forms e.g. poly (anhydride-esters), poly (anhydride-imides), etc. and their potential release characteristics have been evaluated.

Polyamides

Polyamides form another important class of polymers particularly as drug delivery matrices. Polyamides with a structural resemblance to polypeptides are used as matrices for the transport of drugs. Examples include different types of poly (amino acids) such as poly (L-glutamic acid), poly (aspartic acid) are derived from the corresponding natural amino acids. Nakanishi and co-workers have developed a polymeric micelle carrier system consisting of PEG-conjugated doxorubicin: poly (aspartic acid) for the transport of doxorubicin. This carrier system has a highly hydrophobic inner core, and therefore, it can also entrap a useful amount of doxorubicin in addition to the conjugated doxorubicin. It circulated in the blood for a long-time and evaded RES uptake due to the hydrophilic polyethylene glycol outer layer. It was effectively accumulated in the tumour tissue by the EPR effect. The entrapped Doxorubicin was released from the inner core by diffusion and expressed stronger activity than free Doxorubicin against all the tumour lines tested. Li and co-workers have synthesised a novel biodegradable poly (ester amide) derived from

3-morpholine and ε-caprolactone. Increase in morpholine content enhanced water absorption of the polymers *in vitro* degradation data and release profiles of 5-fluorouracil showed that both the degradation rate and drug release rate increased with an enhanced morpholine content in the polymers.

Preparation Techniques

Preparation of nanoparticles can be a variety of different ways. The most important and frequently used is emulsion polymerization, solvent evaporation nanoprecipitation, salting out, and desolvation of natural proteins.

The materials used to prepare nanoparticles are also numerous, but most commonly they are polymers such as poly alkylcyanoacrylates, poly methylmethacrylate, polybutyl cyanoacrylate or from albumin.

For drug loading of nanoparticles, three major strategies can be employed:

(i) Covalent attachment of the drug to the particle surface or to the polymer prior to preparation.

(ii) Adsorption of the drug to a preformed carrier system.

(iii) Incorporation of the drug into the particle matrix during particle preparation.

The release rates of nanoparticles depend upon:

(i) Desorption of the surface- bound/adsorbed drug.

(ii) Diffusion through the nanoparticle matrix.

(iii) Diffusion (in case of nanocapsules) through the polymer wall.

(iv) Nanoparticle matrix erosion.

(v) A combined erosion/diffusion process.

During these preparation and release processes, the bioactivity of therapeutic agent must remain intact. Therefore, the ideal goal would be to achieve satisfactory protein stabilization and appropriate release through a reasonable preparation strategy.

Depending on the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained with different properties and release characteristics for the nanocapsulated therapeutic agent. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a polymer membrane, whereas nanospheres are matrix systems in which the drug is physically and uniformly dispersed (See Fig. 2)

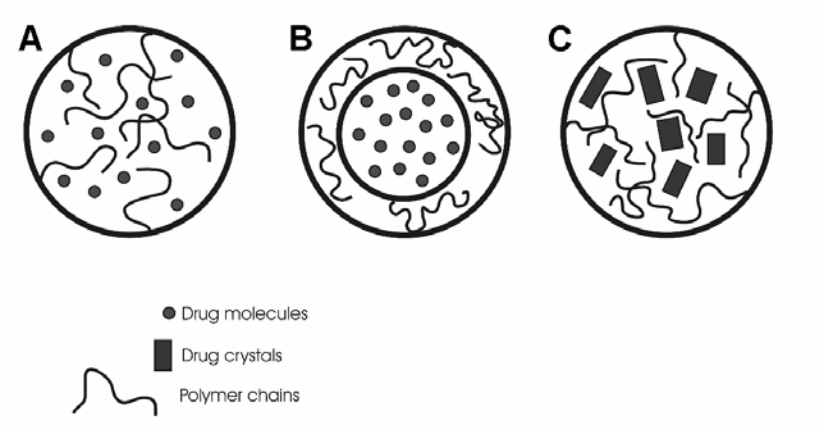


Fig. 2. Schematics of exemplary types of drug nanoparticles.

A. Matrix type nanosphere, drug molecules are evenly dispersed in the polymer matrix.

B. Core shell nanocapsule, drug molecule is presented in a core covered with a polymer shell.

C. Matrix type nanosphere where drug crystals are embedded in a polymer matrix.

1. Solvent displacement

Solvent displacement or nanoprecipitation, also known as the Marangoni effect, has become a popular technique to prepare nanoparticles due to narrow size distribution, absence of shear stress, and absence of surfactants for amphiphilic polymers. This method differs from the emulsification diffusion and salting-out methods in that formally no precursor emulsion is formed during nanoparticle preparation. Basically, nanoparticle formation can be explained in terms of the interfacial turbulence and the “diffusion-stranding” processes between two unequilibrated liquid phases shown in Figure 3. When both phases are in contact, it is assumed that solvent diffuses from the organic phase into the water and carries with it some polymer chains which are still in solution. During the solvent diffuses further into the water, the associated polymer chains aggregate and form nanoparticle shown in Figure 4. The mechanism of nanoparticle formation can be described based on the water-solvent, water-polymer and solvent-polymer interactions. With this technique, PLGA, PCL, SB-PVA-g-PLGA and Methacrylic acid copolymer nanoparticles loaded with therapeutic drugs, e.g. TRH and elcatonin, cyclosporin A were extensively studied. However, the exposure to organic solvent for labile proteins during the preparation process and low encapsulation efficiency for water soluble drugs limit the application of this method.

Fig. 3. Schematic diagram of the mechanism of Marangoni effect

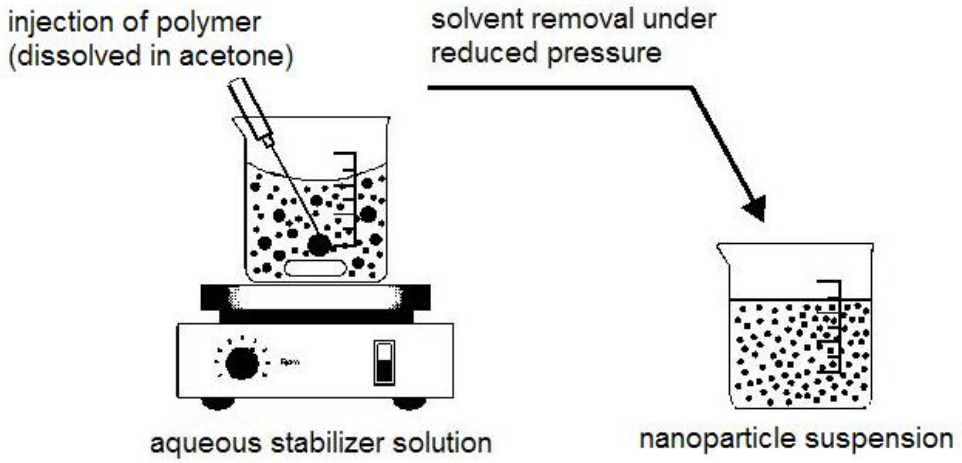


Fig. 4. Schematic diagram of nanoparticles preparation using solvent

displacement method

2. Adsorption process

Stabilization of proteins in delivery devices and design of appropriate protein carriers are major research issues. Preservation of bioactive protein and improvement of drug loading during nanoparticles preparation based on defined colloidal characteristics are a great challenge. Denaturation of protein during preparation primarily is due to high shear forces and solvent exposure; furthermore, high acidity in the nanoparticles matrix due to the polymer degradation also leads to the loss of bioactivity of protein.

Compared to other loading methods, this adsorption technique can be performed in an aqueous solution and at a low temperature, improving the prospects for preserved activity of sensitive drug molecules. Moreover, polymer degradation has no detrimental effect on the protein absorbed on the surface of nanoparticles. However, it is reported that a large amount of drug can be entrapped by the incorporation method when compared to the adsorption. For a successful NP system, a high loading capacity is desirable to reduce the quantity of the carrier required for administration. Many efforts have been made to develop a method to associate the protein to the nanoparticle surface by adsorption. Additionally, a higher

burst release up to 60-70% for the NPs loaded with drug by adsorption. Further efforts related to adsorption process need to be made to investigate the interaction between the protein molecules and surface of NPs, to improve the loading efficiency and to achieve the desired release profile.

3. Surface adsorption on preformed particles with ionic surface charge

An elegant and efficient method for protein loading was done by surface adsorption of bioactive materials onto unloaded PLGA particles carrying a surface charge. One may take advantage of the protein's surface charge, which depends on its pI and the pH of the medium in which it is dispersed. PLGA or any other type of particles can be readily decorated with positive or negative surface charges by simply preparing the particles by $W_1/O/W_2$ solvent evaporation/extraction process where the W_2 phase contains a cationic emulsion stabilizer [hexadecyltrimethyl-ammonium romide; poly (ethyleneimine); stearlyamine] or an anionic emulsifier (sodium dioctyl-sulfosuccintate; sodium dodecylsulfate).

Such compounds attach tightly to PLGA surfaces during preparation and provide the necessary surface charge for ionic adsorption of counter-ions. In these systems, however, the use of chlorinated solvents and high amounts of surfactants, detergents during particle preparation

may affect their biocompatibility, in particular for the development of injectable formulations. A recent approach has been employed using biodegradable polymers carrying cationic or anionic groups, such as sulfobutylated copolymers. Particles made from such polyelectrolytes exposed surface charges, which were used to adsorb oppositely charged protein antigens. Provided that the ionic interaction between the particle surface and the adsorbate does not hamper the activity and availability of the bioactive material, such systems should hold great promise for antigen and DNA delivery. The use of particles with ionic surface charge offers several advantages over classical micro- or nano-encapsulation, amongst which the mild conditions for loading is probably the most attractive. PLGA particles with surface adsorbed protein antigens and DNA have been highly efficient in inducing strong immune responses, as recently reviewed by Singh *et al.,* (2000).

Methods for the characterization of Nanoparticles

Parameters	Methods
Particle size	Scanning electron microscopy (SEM) Transmission electron microscopy (TEM)
Molecular weight	Gel chromatography
Density	Helium compression pycnometry
Crystallinity	X- ray diffraction

Surface charge	Electrophoresis Laser Doppler anemometry
Hydrophobicity	Hydrophobic interaction chromatography
Surface element analysis	X- ray photoelectron spectroscopy for chemical analysis (ESCA)
***In vitro* release**	Biological membranes, Dialysis bag diffusion, Ultra centrifugation, Ultra filtration.

Nanoviricide approach for destruction of viruses:

Research on antiviral nanoparticles has provided clues to the regulation of cytoplasmic transport. Viruses that replicate their genomes in the nucleus make use of the microtubule and the cytoskeleton as molecular motors for trafficking toward the nuclear membrane during entry and the periphery during egress after replication. Analyzing the underlying principles of viral cytosolic transport will be helpful in the design of viral vectors to be used in research as well as human gene therapy, and in the identification of new antiviral target molecules (Dohner and Sodeik., 2005).

Reason for drug delivery of antiviral drugs:

Antiviral drugs are designed with the aim to act on virus-specific processes differing from normal biological events in the host cells. Nevertheless, complete virus specificity is never obtained and manipulation of the whole body disposition may be necessary to obtain a

sufficient therapeutic index.

Features prompting the design of drug targeting formulations for antiviral compounds are:

- Extremely rapid excretion or metabolism, endangering the formation of therapeutic concentrations at practical dosage regimens.
- Severe toxicity in non-target tissue. Examples are bone marrow depression and severe neurotoxicity of Vidarabine (Ara-A) or formation of very toxic metabolites as recently reported for Zidovudine (AZT).
- Poor penetration into the target cells, e.g. Anionic agents that are potent reverse transcriptase inhibitors and anti-herpes drugs that poorly penetrates the Blood Brain Barrier (BBB)**.**
- Inadequate phosphorylation of nucleoside analogues into their active triphosphate forms.
- Poor water solubility leading to limitation in topical administration or precipitation of the drug in the renal tubuli Acyclovir (ACV).

Controlled delivery of Nanoviricide

Controlled delivery of drug is also important. It provides

- Controlled, including linear, release profile.
- Low initial release: no burst.
- Well- preserved compound stability and activity.

- Biodegradable and biocompatible.

Targeted delivery of Nanoviricide

Targeting drugs to specific organs, tissues, or cells is an attractive strategy for enhancing drug efficacy and reducing side effects. Drug carriers such as antibodies, natural and manmade polymers, and labeled liposomes are capable of targeting drugs to extracvascular sites. An alternative strategy is to use low molecular weight prodrugs that distribute throughout the body but cleave intracellularly to the active drug by an organ-specific enzyme.

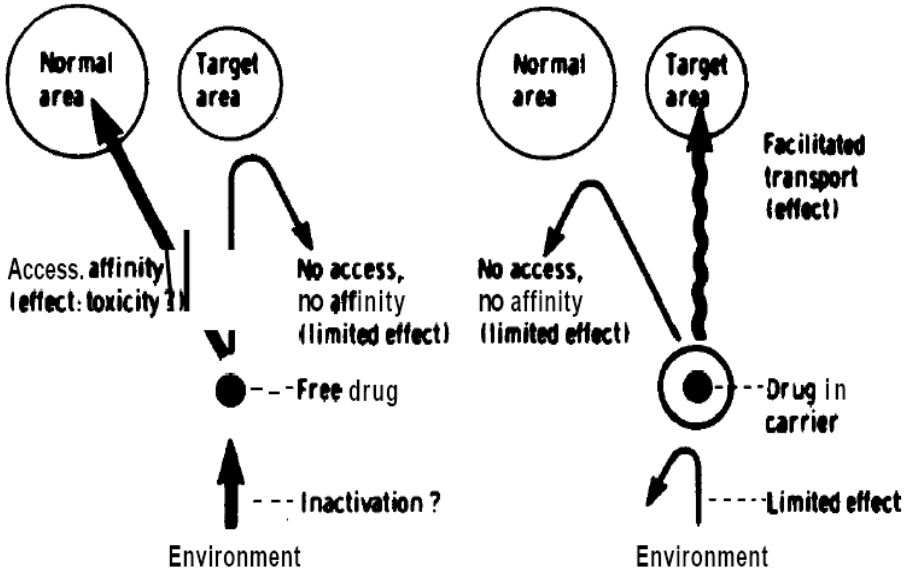


Fig. 5. Principle of drug targeting by carriers

An example of this approach is a series of phosphate and

phosphonate prod rugs, called Hep Direct prod rugs, which enable liver-targeted drug delivery following a cytochrome P450-catalyzed oxidative cleavage reaction inside hepatocytes (Erion *et al.,* 2005) .Glutathione within the hepatocytes rapidly, reacted with the byproduct to form a glutathione conjugate. No byproduct-related toxicity has been observed in hepatocytes or animals treated with HepDirect prodrugs. HepDirect prodrugs represent a potential strategy for targeting drugs to the liver and achieving more effective therapies against chronic liver diseases such as hepatitis B and C.

NANOPARTICLES AS A CARRIER FOR ANTIVIRAL DRUGS

The carrier approach:

The antiviral drug is covalently coupled to soluble macromolecules or included in particle type of drug carrier nanoparticles. The fate of the drug in the body is now dictated by the chosen carrier. The drug concentration in the target tissue is a resultant of relative rates of cellular uptake of the drug conjugate, liberation of the drug as well as efflux rate of free drug from the target tissue. The advantage of targeting is only significant if the target compartment is different from the compartment where toxicity is occurring and if the active drug is released predominantly at the target site.

The Prodrug approach:

Chemical derivatives of the drugs are prepared those has more favourable kinetic features and/or are specifically activated at the site of action.

Preferably the release rate should be controllable

Nanocarriers have important potential applications for the administration of therapeutic molecules. Nanoparticles made from polyhexylcyanoacrylate (PHCA) or human serum albumin (HSA) with a diameter of 200nm were found to be most suitable for targeting of antiviral substances to macrophages. (Hagen von Briesen *et al.,* 1996).

Silver nanoparticles undergo a size-dependent interaction with viral and particles in the range of 1-10 nm attached to the virus (Elechiguerra *et al.,* 2005). The regular spatial arrangement of the attached nanoparticles, the center-to-center distance between nanoparticles, and the fact that the exposed sulfur-bearing residues of the glycoprotein knobs would be attractive sites for nanoparticle interaction suggest that silver nanoparticles interact with the virus via preferential binding to the glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells, as demonstrated *in vitro*. (Hagen von Briesen *et al.,*1996).

APPLICATIONS AND ADVANTAGES OF DRUG CARRIER VEHICLE

Polymeric nanoparticles made from natural and synthetic polymers have received the majority of attention due to higher stability and the opportunity for further surface nanoengineering. They can be tailor-made to achieve both controlled drug release and disease specific localization by tuning the polymer characteristics and surface chemistry. It has been established that nanocarriers can become concentrated preferentially in the tumour mass, inflammatory sites, and at infectious sites by virtue of the enhanced permeability and retention (EPR) effect of the vasculature. Once accumulated at the target site, hydrophobic biodegradable polymeric nanoparticles can act as a local drug depot depending upon the make-up of the carrier, thus providing a source for a continuous supply of encapsulated therapeutic compound at the disease site, such as, a solid tumour. These systems in general can be used to provide targeted (cellular/tissue) delivery of drugs, to improve oral bioavailability, to sustain drug/gene effect in the target tissue, to solubilize drugs for intravascular delivery, and to improve the stability of therapeutic agents against enzymatic degradation (nucleases and proteases), especially of protein, peptide, and nucleic acids drugs. Thus, the advantages of using nanoparticles for drug delivery result from two main basic properties viz. small size and use of biodegradable materials. Nanoparticles, because of their small size, can extravasate through the sites of viral inflammation and cancer cells or penetrate into smaller capillaries, cross the fenestration

present in the epithelial lining (e.g., liver), and are generally taken up efficiently by the cells which allow efficient drug accumulation at the target sites. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system. Nanoparticles have a further advantage over larger microparticles as they are better suited for intravenous (i.v.) delivery. The smallest capillaries in the body are 5–6 μm in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5 μm, without forming aggregates to ensure that the particles do not form an embolism. In some cell lines, only submicron nanoparticles can be taken up but not large size microparticles. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and are available to a much wider range of biological targets due to their small size and relative mobility. The 100 nm nanoparticles had a 2.5 fold greater uptake than 1 μm microparticles, and 6 fold greater up take than 10 μm microparticles in a Caco-2 cell line. In a similar study, the nanoparticles penetrated throughout the submucosal layers in a rat in situ intestinal loop model, while microparticles were predominantly localized in the epithelial lining. Secondly, the use of biodegradable materials for nanoparticle preparation allows sustained drug release within the target site over a period of days or even weeks. Biodegradable nanoparticles formulated from poly D, L-lactide co-glycolide (PLGA) and Polylactide (PLA) have

been developed for intracellular sustained drug delivery, especially for drugs with an intracellular target. Rapid escape of PCL nanoparticles from the endo-lysosomal compartment to the cytoplasmic compartment has been demonstrated. Thus, nanoparticles could be an effective drug delivery mechanism for drugs whose targets are cytoplasmic. Greater and sustained antiproliferative activity was observed in vascular smooth muscle cells which were treated with dexamethasone-loaded nanoparticles compared to that seen with drug in solution. Nanoparticles were effective in sustaining intracellular dexamethasone levels, thus allowing a more efficient interaction with the glucocorticoid receptors which are cytoplasmic.

HSV2 AND HIV

HSV2

Herpes simplex virus type 2 (HSV2) infection is the primary cause of genital herpes. It is highly prevalent in human populations in many parts of the world, and is the most common cause of genital ulcer disease worldwide. In developing countries, the major public health importance of HSV2 relates to its potential role in facilitating HIV transmission. HSV2 is highly prevalent in most regions experiencing severe HIV epidemics, with infection rates rising steeply with age to reach levels of 70% or more among adult women and men in some African countries. Genital ulcer

disease enhances the infectiousness of HIV-positive subjects and the susceptibility of HIV-negative subjects, and clinical research has shown effects of HSV2 infection on genital HIV shedding. The reciprocal effect of HIV immune suppression on the exacerbation of HSV2 symptoms implies that there is a positive feedback loop, with HIV enhancing HSV2 expression, which in turn may enhance HIV infectiousness and its spread. Accumulating data suggest that HSV2 may be responsible for a substantial proportion of new HIV infections in some parts of Africa. There is an urgent need to consider potential control measures for HSV2 that might be applied in an effort to curb HIV transmission. These might include episodic or suppressive antiviral therapy, for example among high-risk groups, and behavioral interventions designed to reduce herpes transmission. Candidate HSV2 vaccines and vaginal microbicides are also under development. An increasing proportion of genital ulcer cases in Africa are now attributable to HSV2, and the implications for treatment algorithms also need to be considered. Given the increasing awareness of the link between HSV2 and HIV, international technical workshops existing knowledge concerning the epidemiology and control of HSV2 in developing countries and its interaction with HIV. While the main focus was on developing countries, where the public health burden of HSV2 is greatest, experts from industrialized countries were also invited to share

perspectives from these countries, where much previous research has been conducted.

EPIDEMIOLOGY AND NATURAL HISTORY OF HSV2

Global epidemiology of HSV2

HSV2 prevalence is increasing worldwide and the major cause of genital ulcer disease (GUD) in the developed world. In the developing world, the major public health importance of HSV2 lies in its potential role as a co-factor for HIV transmission. The high prevalence of HSV2 in many populations results from the fact that it is a lifelong infection, which is highly infectious and often transmitted in the absence of symptoms. Overall, prevalence is higher in women compared with men, especially among the young, and rates of up to 40% have been recorded among women aged 15-19 in Kisumu, Kenya. Infection has been associated with younger age at first sex, increased years of sexual activity, increasing number of lifetime partners, lack of circumcision (in men) and current or recent other STIs.

HIV

HIV stands for Human Immunodeficiency Virus, the virus that causes AIDS.

H- Human

I – Immunodeficiency

V – Virus

- HIV breaks down the body's defence against infection and disease

– the body's immune system by infecting specific white blood cells, leading to a weakened immune system.

- When the immune system becomes weak or compromised, the body loses its protection against illness.
- As time passes, the immune system is unable to fight the HIV infection and person may develop serious and deadly, including other infections and some types of cancer.

AIDS is an acronym for acquired immunodeficiency syndrome and refers to the most advanced stage of HIV infection.

A – **A**cquired, (not inherited) to differentiate form a genetic or inherited condition that causes immune dysfunction.

I – **I**mmune, because it attacks the immune system and increases susceptibility to infection.

D – **D**eficiency of certain white blood cell in the immune system.

S - **S**yndrome, a group of symptoms or illnesses that result from the HIV infection.

Differences between HIV, HIV Infection, and AIDS

- HIV is the virus that causes infection.
- The person who is HIV- infected may have no signs of illness but can still infect others.
- Most people who are HIV- infected will develop AIDS after a period of time, which may be several months to more than 15 years.

- AIDS is a group of serious illnesses and opportunistic infections that develop after being infected with HIV for a long period of time.
- A diagnosis of AIDS is based on specific clinical criteria and laboratory test results.

Types of HIV

HIV-1 and HIV-2 are types of HIV. Both types are transmitted the same way, and both are associated with similar opportunistic infections and AIDS. HIV-1 is more common worldwide. HIV-2 is found predominantly in West Africa, Angola, Mozambique.

Differences between HIV-1 and HIV-2

HIV-2 is less easily transmitted than HIV-1, and it is less pathogenic, meaning that the period between initial infection and illness is longer. In some areas, a person may be infected with both HIV-1 and HIV-2. While HIV-2 can be transmitted from an infected mother to her child, this appears to be rare (0% to 5% transmission rate in breastfed infants in the absence of any interventions).

INTERACTION BETWEEN HSV2 AND HIV

Biological and Clinical Research

HIV and HSV2 manifest a bi-directional interaction. HSV2 increases the efficiency of HIV acquisition and transmission whereas HIV may increase susceptibility to HSV2 and increase HSV2 shedding, HSV2

recurrence rate and severity of clinical manifestations.

Effect of HIV infection on natural history of HSV2

HSV2 reactivation and duration of recurrences are significantly increased in HIV infected individuals. The frequency and severity of recurrences increases as CD4 cell count decreases.

Effect of HIV infection on HSV2 transmission

HIV infection is also likely to increase transmission of HSV2, as there is evidence that the prevalence and quantity of genital HSV2 shedding is significantly increased among HIV seropositive individuals.

Effect of HSV2 on natural history of HIV disease

There is some evidence that inclusion of Valacyclovir in antiretroviral therapy may prolong survival in HIV seropositive individuals. One study has shown that HSV2 reactivation is associated with increases in plasma HIV1 RNA and intracellular gag mRNA and that plasma HIV1 RNA level decreases significantly during treatment with Valacyclovir. As a result, it is possible that HIV progresses more rapidly in untreated HSV2 positive individuals. However, evidence is inconclusive and more studies of the effect of episodic HSV2 therapy on HIV are needed, especially in developing countries.

Effect of HSV2 on HIV transmission

A study of 12 men in the US infected with both HSV2 and HIV

showed that HIV RNA was present in almost all HSV2 lesions, suggesting that HIV transmission is enhanced in the presence of HSV2 lesions. HIV RNA is often present at high titres in genital lesions (independent of plasma RNA levels), but titres of both HIV1 and HSV2 fall rapidly on treatment with Valacyclovir, supporting the hypothesis that HSV2 reactivation may play an important role in regulation of HIV1 on mucosal surfaces. Few data are available on the effect of subclinical HSV2 on HIV shedding and viral load, or on the relative effects of symptomatic and subclinical infection. It is plausible that the interaction between the two viruses differs in developed and developing countries, due to the role of other factors (such as circumcision, prevalence of other STIs and other tropical conditions or infections), and similar prospective studies of dually-infected individuals are needed in developing countries. Further biological studies are also needed to define the mechanism by which HIV is secreted on the mucosal surface.

MACROPHAGE

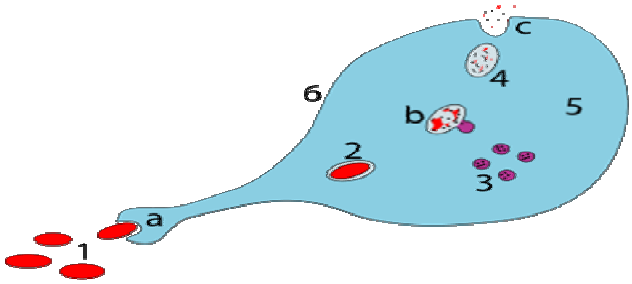
Greek: big eaters, from *makros* "large" + *phagein* "eat"; are white blood cells within tissues, produced by the division of monocytes. Human macrophages are about 21 microns in diameter. Monocytes and macrophages are phagocytes, acting in both non-specific defense (and innate immunity) as well as to help initiate specific defense mechanisms (or cell-mediated immunity) of vertebrate animals. Their role is to

phagocytose (engulf and then digest) cellular debris and pathogens either as stationary or mobile cells, and to stimulate lymphocytes and other immune cells to respond to the pathogen.

Life cycle

When a monocyte enters damaged tissue through the endothelium of a blood vessel (a process known as the leukocyte extravasation), it undergoes a series of changes to become a macrophage. Monocytes are attracted to a damaged site by chemical substances through chemotaxis, triggered by a range of stimuli including damaged cells, pathogens and cytokines released by macrophages already at the site. At some sites such as the testis, macrophages have been shown to populate the organ through proliferation. Unlike short-lived neutrophils, macrophages survive longer in the body up to a maximum of several months.

Function



Parts

1. Pathogens

2. Phagosome

3. Lysosomes

4. Waste material

5. Cytoplasm

6. Cell membrane

Steps of a macrophage ingesting a pathogen

a. Ingestion through phagocytosis, a phagosome is formed

b. The fusion of lysosomes with the phagosome creates a phagolysosome; the pathogen is broken down by enzymes

c. Waste material is expelled or assimilated (the latter not pictured)

Role in specific immunity

Macrophages are versatile cells that play many roles. As scavengers, they rid the body of worn-out cells and other debris. They are

foremost among the cells that "present" antigen; a crucial role in initiating an immune response. As secretory cells, monocytes and macrophages are vital to the regulation of immune responses and the development of inflammation; they produce an amazing array of powerful chemical substances (monokines) including enzymes, complement proteins, and regulatory factors such as interleukin-1. At the same time, they carry receptors for lymphokines that allow them to be "activated" into single-minded pursuit of microbes and tumour cells.

After digesting a pathogen, a macrophage will present the antigen (a molecule, most often a protein found on the surface of the pathogen, used by the immune system for identification) of the pathogen to the corresponding helper T cell. The presentation is done by integrating it into the cell membrane and displaying it attached to a MHC class II molecule, indicating to other white blood cells that the macrophage is not a pathogen, despite having antigens on its surface.

Eventually the antigen presentation results in the production of antibodies that attach to the antigens of pathogens, making them easier for macrophages to adhere to with their cell membrane and phagocytose. In some cases, pathogens are very resistant to adhesion by the macrophages.

The antigen presentation on the surface of infected macrophages

(in the context of MHC class II) in a lymph node stimulates TH1 (type 1 helper T cells) to proliferate (mainly due to IL-12 secretion from the macrophage). When a B-cell in the lymph node recognizes the same unprocessed surface antigen on the virus with its surface bound antibody, the antigen is endocytosed and processed. The processed antigen is then presented in MHCII on the surface of the B-cell. TH1 receptor that has proliferated recognizes the antigen-MHCII complex (with co-stimulatory factors- CD40 and CD40L) and causes the B-cell to produce antibodies that help opsonisation of the antigen so that the virus can be better cleared by phagocytes.

Macrophages provide yet another line of defense against tumor cells and somatic cells infected with fungus or parasites. Once a T cell has recognized its particular antigen on the surface of an aberrant cell, the T cell becomes an activated effector cell, chemical mediators known as lymphokines that stimulate macrophages into a more aggressive form. These activated macrophages can then engulf and digest affected cells much more readily. The macrophage does not generate a response specific for an antigen, but attacks the cells present in the local area in which it was activated.

Macrophage

Name of cell	Location
Dust cells/Alveolar macrophages	Pulmonary alveolus of lungs
Histiocytes	Connective tissue
Kupffer cells	Liver
Microglia	Neural tissue
Epithelioid cells	Granulomas
Osteoclasts	Bone
Sinusoidal lining cells	Spleen
Mesangial cells	Kidney

Involvement in symptoms of diseases

Due to their role in phagocytosis, macrophages are involved in many diseases of the immune system. For example, they participate in the formation of granulomas, inflammatory lesions that may be caused by a large number of diseases. Some disorders, mostly rare, of ineffective phagocytosis and macrophage function have been described. Macrophages are the predominant cells involved in creating the progressive plaque lesions of atherosclerosis. Macrophages also play a role in Human Immunodeficiency Virus (HIV) infection. Like T cells, macrophages can be infected with HIV, and even become a reservoir of

ongoing virus replication throughout the body. Macrophages are believed to help cancer cells proliferate as well. They are attracted to oxygen-starved (hypoxic) tumour cells and promote chronic inflammation. Inflammatory compounds such as Tumor necrosis factor (TNF) released by the macrophage activates the gene switch nuclear factor-kappa B. NF-kB then enters the nucleus of a tumour cell and turns on production of proteins that stop apoptosis and promote cell proliferation and inflammation.

LITERATURE REVIEW

Stieneker *et al.,* (2008) Methylmethacrylate (MMA) sulfopropyl-methacrylate (SPM) copolymer nanoparticles were prepared by free radical polymerization. The conditions of preparation were varied with regard to the concentration of initiator and monomer, and copolymer composition. Nanoparticles with a yield greater than 80% were produced. The particles were characterized in terms of particle size, size distribution, particle charge (zetapotential) and molecular weight. The data were compared to pure polymethylmethacrylate (PMMA) nanoparticles. The copolymer composition was shown to influence particle size and particle charge. The influence of the total monomer amount in the polymerization medium on the particle size was characteristic up to a concentration of 2% depending on the solubility of MMA in water at the temperature of reaction. An increasing amount of total monomer led to particle sizes of 60-130 nm for low monomer concentrations (0.5%), depending on the proportion of SPM (0-10%), to 120-280 nm for higher total monomer concentrations (greater than 2%). Surface charge as well as particle size was influenced mainly by the proportion of the comonomer SPM in the copolymer. The negative surface charge increased from - 52 mV for pure PMMA nanoparticles to - 80 mV for the copolymer particles with an SPM content of 10%. In the same range of 10% SPM of the total monomer, the particle

sizes decreased from 187 to 100 nm. The concentration of the initiator up to a concentration of 0.3% showed no effect on the particle size of the resulting nanoparticle suspension. Higher concentrations led to intolerably large variability in the polymerization process.

Narendra Jain *et al.,* (2008) HIV infected macrophages are considered as reservoirs for spreading the virus in AIDS patients. Tuftsin not only binds specifically to the mononuclear phagocytic cells but also enhances their natural killer activity. The purpose of this study is to explore the targeting potential and anti-HIV activity of Efavirenz (EFV) loaded, Tuftsin conjugated 5th generation poly (propyleneimine) dendrimers (TuPPI) *in vitro*. Tuftsin was chemically conjugated to 5^{th} generation poly (propyleneimine) dendrimers (PPI). The entrapment efficiency of PPI and TuPPI were found to be 37.43±0.3% and 49.31±0.33%, respectively. TuPPI was found to slow down and prolong the *in vitro* release of EFV upto 144h against PPI, which releases the drug completely within 24 hr. TuPPI possessed negligible cytotoxicity as compared to that of PPI. The cellular uptake of TuPPI was found to be 34.5 times higher than that of the free drug in first 1hr and was significantly higher in HIV infected macrophages than that of uninfected cells. TuPPI was found to reduce the viral load by 99% at a concentration of 0.625ng/ml, which is due to the enhanced cellular uptake, reduced toxicity and the inherent anti-HIV activity of TuPPI.

Davi Pereira de Santana *et al.,* (2007) We report the development and validation of a new sensitive, accurate and precise HPLC method with ultraviolet detection for the determination of Indinavir sulfate (IND) in human plasma and its application to a bioequivalence study of a new generic formulation. The extraction of IND from plasma samples was achieved by using liquid-liquid extraction with a mean recovery of 73.9%. The lower limit of quantification was 0.05μg/mL. Bioequivalence between the products was determined by calculating 90% confidence intervals (CI) for the ratio of C_{max}, AUC_{0-t} and AUC_{0-inf} values for the test and reference products, within the 0.80-1.25 interval proposed by ANVISA and FDA. Therefore the medications are bioequivalent and inter-exchangeable.

Yung-Chih Kuo *et al.,* (2007) Permeability of the anti-human immunodeficiency virus (HIV) agents, including Stavudine (D4T), Delavirdine (DLV), and Saquinavir (SQV), across the in vitro blood–brain barrier (BBB) was studied. Here, the anti-HIV agents were incorporated withpolybutylcyanoacrylate (PBCA) nanoparticles (NPs), methylmethacrylate- sulfopropylmethacrylate (MMA-SPM) NPs, and solid lipid nanoparticles (SLNs). Transport of the anti- HIV agents across BBB is a key factor in their applications to the therapy of the acquired immunodeficiency syndrome (AIDS). Experimental results revealed that

the drug order of the loading efficiency (LE) on PBCA and MMA-SPM was D4T >DLV> SQV. For the entrapment efficiency (EE) in SLNs, this order was reversed. Also, LE of D4T on MMA-SPM was larger than that on PBCA; however, the reverse was true for DLV and SQV. As the particle size increased, LE decreased and EE increased. For a fixed drug carrier, an increase in the particle size yielded a decrease in the BBB permeability coefficient of the anti-HIV agents. Moreover, enhancement in the BBB permeability was on the carrier order of PBCA >MMA-SPM> SLNs for D4T, and for DLV and SQV, the order became PBCA > SLNs > MMA-SPM. PBCA, MMA-SPM, and SLNs were efficacious carriers of D4T, DLV, and SQV to meliorate BBB permeability by 3–16 folds, indicating the clinical potential of the present NP formulations for the AIDS treatment.

Lipa Shah *et al.,* (2006) Aim to developing poly (ethylene oxide) - modified poly (epsilon-caprolactone) (PEO-PCL) nanoparticulate system as an intracellular delivery vehicle for Saquinavir**,** an anti-HIV protease inhibitor. The PEO-PCL nanoparticles had a smooth surface and spherical shape and showed a relatively uniform size distribution with a mean particle diameter of approximately 200nm. Intracellular Saquinavir concentrations when administered in the nanoparticle formulation were significantly higher than from aqueous solution. PEO-PCL nanoparticles provide a versatile platform for encapsulation of Saquinavir and

subsequent intercellular delivery in Mo/Mac cells.

Gladys Granero *et al.,* (2006). The absolute bioavailability of the prodrug Valacyclovir, the l-Valyl ester of acyclovir, after oral administration is 54.5%. Since premature hydrolysis of this prodrug in the intestinal lumen may be a possible reason for its incomplete bioavailability and the chemical and enzymatic stability of the Valacyclovir has been investigated. Release rates were investigated in both phosphate buffers with varying pH as well as in human and dog gastrointestinal fluids. The stability of the prodrug was found to be dependent on pH. This prodrug is chemically stable along the acidic pH side (under 4), while the prodrug degrades in alkaline medium through a base-catalyzed pseudo-first-order kinetics. The degradation of the prodrug Valacyclovir progressed faster in intestinal fluid than in phosphate buffer at the same pH. There was no appreciable release of Valacyclovir neither in the human and dog stomach contents nor in phosphate buffers at pHs fewer than 4, although its degradation was fastest in the human and dog stomach contents. In light of this result, we can conclude that the degradation of the Valacyclovir in the upper intestinal lumen is probably one of the causes of its poor bioavailability.

Ghania Degobert *et al.,* (2006). Freeze-drying has been considered as a good technique to improve the long-term stability of colloidal nanoparticles. The poor stability in an aqueous medium of these

systems forms a real barrier against the clinical use of nanoparticles. This article reviews the state of the art of freeze-drying nanoparticles. It discusses the most important parameters that influence the success of freeze-drying of these fragile systems, and provides an overview of nanoparticles freeze-drying process and formulation strategies with a focus on the impact of formulation and process on particle stability.

Yung-Chih Kuo *et al.,* (2006). Effect of size of nanoscaled polybutylcyanoacrylate (PBCA) and methylmethacrylate–sulfopropylmethacrylate (MMA–SPM) on the permeability of Zidovudine (AZT) and Lamivudine (3TC) across the blood–brain barrier (BBB) was investigated. Also, influence of alcohol on the permeability of AZT and 3TC incorporated with the two polymeric nanoparticles (NPs) was examined. The loading efficiency and the permeability of AZT and 3TC decreased with an increase in the particle size of the two carriers. By employing PBCA NPs, the BBB permeability of AZT and that of 3TC became, respectively, 8–20 and 10–18 folds. Application of MMA–SPM NPs leaded to about 100% increase in the BBB permeability of the two drugs. In the presence of 0.5% ethanol, 4–12% enhancement in the BBB permeability of the two drugs was obtained in the current carrier-mediated system.

Yiguang Jin *et al.,* (2006). Self-assembled drug delivery systems (SADDS) were designed in the paper. They can be prepared from the

amphiphilic conjugates of hydrophilic drugs and lipids through self-assembling into small-scale aggregates in aqueous media. The outstanding characteristic of SADDS is that they are nearly wholly composed of amphiphilic prodrugs. The self-assembled nanoparticles (SAN) as one of SADDS had been prepared from the lipid derivative of acyclovir (SGSA) in the previous paper. They were further studied on the properties and the in vitro/in vivo behavior in this paper. The SAN kept the physical state stable upon centrifugation or some additives including some inorganic salts, alkaline solutions, surfactants and liposomes except for HCl solution, CaCl2 solution and animal plasma. Autoclave and bath heat for sterilization hardly influenced the SAN. However, gamma-irradiation strongly destroyed the structure of SAN and SGSA was degraded. SGSA in SAN showed good stability in weak acidic or neutral buffers although it was very sensitive to alkaline solutions and Carboxyl ester enzymes, the half-lives ($t_{1/2}$) of which in the buffer at pH 7.4, the alkaline solution at pH 12.0, pig liver carboxylester enzyme solution, rabbit plasma, and rabbit liver tissue homogenate were 495, 21, 4.7, 25 and 8.7 h, respectively. Compared with SGSA in a disordered state, the specific bilayer structures of SAN could protect SGSA from hydrolysis through hiding the sensitive ester bonds. The SAN showed hemolytic action because the amphiphilic SGSA could insert into rabbit erythrocyte membranes. Both the high concentration of SGSA in samples and the long incubation time improved

hemolysis. No hemolysis was observed if the additional volume of the SAN was less than 10% of rabbit whole blood in spite of the high concentration of SGSA. Plasma proteins could interfere the interaction between the SAN and erythrocytes by binding the SAN. The in vitro antiviral activity of acyclovir SAN was limited possibly because of the weak hydrolysis of SGSA in Vero cells, and the SAN showed a little cell toxicity possible due to the amphiphilicity of SGSA. A macrophage cell line of QXMSC1 cells showed uptake of the SAN but not significantly. The SAN were rapidly removed from blood circulation after bolus i.v administration to rabbits with the very short distribution $t_{1/2}$ (1.5 min) and the elimination $t_{1/2}$ (47 min). The SAN were mainly distributed in liver, spleen and lung after i.v administration, and SGSA was eliminated slowly in these tissues ($t_{1/2}$, about 7 h). It would appear that the nanosized SAN were trapped by the mononuclear phagocyte system. SADDS including SAN combine prodrugs, molecular self-assembly with nanotechnology, and hopefully become novel drug delivery approaches.

Yung-Chih Kuo *et al.,* (2005). Loading efficiency (LE) of Stavudine (D4T), a human immunodeficiency antiretroviral agent, on the external surfaces of Polybutylcyanoacrylate (PBCA) and Methylmethacrylate-Sulfopropylmethacrylate (MMA-SPM) was investigated. The experimental results indicate that the larger the polymeric nanoparticles (NPs), the smaller LE of D4T on the two kinds of biomaterials. Freeze drying of the

two NPs, however, yields an increase in particle size and an increase in LE of D4T, in general. Preservation of the two D4T-loaded NPs through cold storage at 4 ◦C over 6 weeks leads to an increase in particle size and a decrease in LE ofD4T. LE of D4T on both of the two NPs decreases with a variation in pH value from pH 7.2 of loading medium. LE of D4T on MMA-SPM NPs is larger than that on PBCA NPs at pH 7.4; and for the case of variation in pH value of loading medium from pH 7.2, the extent of decrease in LE of D4T for MMA-SPM NPs is higher than that on PBCA NPs. These outcomes imply that for oral administration, D4T-loaded MMA-SPM NPs may be more advantageous than D4T-loaded PBCA NPs, and D4T-loaded PBCA NPs may be more favorable than D4T-loaded MMA-SPM NPs for intravenous injection.

Bergshoeff *et al.,* (2004). So far, no pediatric doses for Indinavir combined with Ritonavir have been defined. This study evaluated the pharmacokinetics of 400 mg of Indinavir/m2 combined with 125 mg of Ritonavir/m2 every 12 h (q12h) in 14 human immunodeficiency virus type 1-infected children. The area under the concentration-time curve from 0 to 24 h and the minimum concentration of drug in serum for Indinavir were similar to those for 800 mg of indinavir–100 mg of Ritonavir q12h in adults, while the maximum concentration of drug in serum was slightly decreased, with geometric mean ratios (90% confidence intervals in parentheses) of

1.1 (0.87 to 1.3), 0.96 (0.60 to 1.5), and 0.80 (0.68 to 0.94), respectively.

Norbert Dinauer *et al.,* (2004). Aim to study about intracellular tracking of protamine / anisense oligonucleotide nanoparticles in inhibitory effect on HIV-1 transactivation. Membrane transport of antisense oligonucleotide (AS-ODN) is a special carrier for intracellular delivery. They have developed a delivery system for AS-ODN and their phosphorothioate analogues (AS-PTO) directed against HIV-1 tat mRNA for efficient transfection of HIV-1 target cells. These results demonstrate that protamine/AS-ODN nanoparticles are useful for future therapeutical application to inhibit viral gene expression.

Sibel Ozkan *et al.,* (2003). A specific, sensitive, simple, and rapid HPLC method has been developed for the determination of valacyclovir (VACL) in raw material, pharmaceutical dosage forms, and human serum, in order to carry out drug dissolution studies from tablets. The chromatographic separation was achieved with acetonitrile: methanol: 0.067M KH_2PO_4 (27:20:53, v/v/v) adjusted to pH 6.5 with 3M NaOH as mobile phase, a Waters Spherisorb CIS column, and UV detection at 244nm. Etodolac was used as an internal standard. Linearity range was 5-20,000 ngmL. Limit of detection obtained was 0.38 and 0.14ngmL in mobile phase and spiked human serum samples, respectively. The described method can be readily applied, without any interference from the excipients, for the determination of the drug in tablets, human serum

samples, and drug dissolution studies.

Veronique Joly *et al.,* (2002). We compared the efficacy and the toxicity of Zidovudine (AZT) versus Stavudine (d4T), in combination with Lamivudine (3TC) and Indinavir, in AZT-, dideoxyinosine (ddI)-,and/or dideoxycytosine (ddC)- experienced patients in a randomized comparative multicenter trial. One hundred seventy human immunodeficiency virus type 1 (HIV-1)-infected patients, who had received AZT, ddI, and/or ddC for at least 6 months but were naïve for d4T, 3TC, and protease inhibitors, were randomized to AZT at 250 to 300 mg twice daily, 3TC at 150 mg twice daily, and Indinavir at 800 mg every 8 h or to d4T at 40 mg twice daily, 3TC at 150 mg twice daily, and Indinavir at 800 mg every 8 h. The primary endpoint was time to virological failure, defined as plasma HIV-1RNA levels of >5,000 copies/ml after at least 8 weeks of antiretroviral therapy. Additional endpoints were change from baseline in CD4 cell counts, AIDS-defining events and adverse events, and proportion of patients with HIV-1 RNA levels of <500 copies/ml and HIV-1 RNA levels of <50 copies/ml. At week 80, 15 patients in the AZT arm and 14 patients in the d4T arm had reached the primary endpoint, and time to virological failure did not differ between the two arms (P - 0.98). In the d4T and in the AZT arms, 67 and 73% of patients, respectively, had HIV-1 RNA levels of <500 copies/ml (P - 0.50). The median change from baseline in CD4 cell count was 195 -106 and 175 - 106/liter for the d4T- and AZT-containing arms, respectively.

The proportions of patients with HIV-1 RNA levels of <50 copies/ml at weeks 8, 16, and 24 were similar in the two arms. The occurrence of serious adverse events was not significantly different between arms. In conclusion, in these patients heavily pretreated with AZT, switching from AZT to d4T when initiating Indinavir and 3TC did not bring any additional benefit compared to maintaining AZT.

Juan Irache *et al.,* (2001). Ganciclovir is one of the most widely used antiviral drugs for the treatment of cytomegalovirus retinitis. Due to its short half-life in the vitreous, frequent administrations are necessary to maintain the therapeutic levels. In this context, the aim of this study was to characterize and in vitro evaluate the drug release properties of three different formulations of Ganciclovir-loaded albumin nanoparticles.These carriers were prepared by a coacervation method and chemical cross-linking with glutaraldehyde. Depending on the step where the drug and/or cross-linking agent were added three different formulations were obtained, named models A, B and C. For model A nanoparticles, Ganciclovir was incubated with the just-formed albumin nanoparticles. For the other two types of nanoparticulate formulations, the drug was added to a solution of albumin (model B) and glutaraldehyde (model C) prior the formation of the carriers by coacervation. In all cases, the size of the different nanoparticulate formulations was comprised between 200 and 400 nm and the yield ranged from 50%, in model A, to 65% in model B. Concerning the Ganciclovir loading, model B nanoparticles offered the higher capacity to

carry this antiviral drug (around 30 mg Ganciclovir /mg nanoparticle). On the contrary, the drug loading calculated for model A nanoparticles was only 14.6 mg/mg. The in vitro release profiles of the nanoparticles showed a biphasic pattern, with an initial and rapid release, followed by a slower step for up 5 days. This burst effect was especially relevant in model A (around 60% in 1 h), followed by model B (40%) and less important in model C (20%). The addition of trypsin to the release medium did not have a significant influence on the release characteristics. However, the release of the drug was increased in acidic or basic mediums, due to the disruption of the covalent binding between Ganciclovir and the protein matrix via glutaraldehyde. This strong linkage was also confirmed by TLC experiences. In summary, a first step of incubation between the drug and the protein, prior the preparation of nanoparticles, enabled us to obtain albumin carriers able to release Ganciclovir in a sustained way.

Uwe wintergerst *et al.,* (2000). Indinavir concentrations were determined in plasma and saliva over a random period of 4 h. On average, levels in saliva were 70%, 38% of the corresponding levels in plasma. These findings suggest that saliva might serve as an appropriate specimen for monitoring of plasma Indinavir levels in patients treated with Indinavir.

Zhang Zhi-Rong et al., (1999) To prepare Valaciclovir polybutylcyanoacrylate nanoparticles (VACV-PBCA-NP) with liver targeting and hepatocyte permeable characteristics. Emulsion

polymerization method was employed to prepare VACV-PBCA-NP. The formula and preparation conditions were optimized by using the uniform design. The organ distribution of the intravenously injected VACV-PBCA-NP and VACV in animal was determined using HPLC. The hepatocytes permeability of VACV-PBCA-NP was demonstrated by cell uptake experiment in vitro. The drug loading and the drug embedding ratio of VACV-PBCA-NP were 11.20% and 84.85% respectively, with an average diameter of 10 4.77nm±11.78nm. The releasing characteristics in vitro fitted the two-phase kinetics. 74.49% of the drug was found to localize in the liver 15min after the administration of VACV-PBCA-NP in the mice. Compared with VACV, VACV-PBCA-NP showed distinct characteristic of sustained-release in vivo and the drug entering hepatocytes were also greatly increased. VACV-PBCA-NP has the characteristic of liver targeting and can increase the permeability of VACV to hepatocytes.

Nigel Phillips *et al.,* (1998). Solid lipid nanoparticles (SLNs) were prepared using trilaurin as the SLNs solid core and a mixture of neutral and negatively charged phospholipid. To produce SLNs with a poly (ethylene glycol) (PEG) coating, PEG was incorporated in SLNs using dipalmitoyl- phosphatidylethanolamine-*N*-[poly (ethylene glycol) 2000] (PE-PEG). 3%-azido-3% deoxythymydine palmitate (AZT-P) with [3H]-AZT-P as tracer were synthesized and incorporated in SLNs. Their subsequent

retention in SLNs with and without PEG was determined after incubation in 50% bovine plasma. Biodistribution studies were performed in mice using free AZT-P, AZT-P incorporated in SLNs or AZT-P incorporated in PE-PEG coated SLNs (SLN-PE-PEG). The presence of PE-PEG significantly reduced the SLN zeta potential from -22 to -5 mV. Although AZT-P was rapidly released from SLNs during incubation in bovine plasma, the release rate was significantly slower in SLN-PE-PEG. AZT-P was rapidly removed from blood following i.v. injection in mice. The decrease in AZT-P blood level was biphasic and rapid, and the major excretory route of AZT-P was the kidney. Higher levels were observed after i.v. injection of AZT-P incorporated in SLNs. This effect was further increased using SLN-PE-PEG. Both SLN and SLN-PE-PEG incorporation of AZT-P significantly decreased the urinary excretion of AZT-P and increased the localization of AZT-P in the liver. The results obtained in this study indicate that using SLNs as a drug carrier increases the bioavailability of incorporated AZT-P, and that the pharmacokinetic behaviour of the incorporated drug can be modified by changing the surface characteristics of SLNs by using the amphiphilic solvation enhancer PE-PEG.

Oh *et al.,* (1998). The effect of current, its magnitude and

penetration enhancers (propylene glycol / oleic acid) on the transdermal flux of AZT (Zidovudine) across hairless mouse skin was studied and the results were compared. The in vitro iontophoretic flux from AZT solution increased to about 5–40 fold that obtained by passive diffusion, depending on the magnitude of current density. When the donor side was karaya gum matrix, instead of solution, the flux enhancement effect by iontophoresis was much smaller. Incorporation of penetration enhancers into the matrix increased the passive flux 2–50 fold, depending on the amount of penetration enhancers in the matrix. These enhancers worked synergistically with iontophoresis in the transdermal transport: a much larger flux than that expected from a simple additive effect was observed. Electrical resistance data from our previous work is utilized to further discuss this synergistic effect.

Kreuter *et al.,* (1997). Reticuloendothelial cells play an important role in the immunopathogenesis of AIDS. For this reason, a targeted delivery of antiviral drugs to these cells should significantly improve therapy of AIDS. The objective of the present study was to investigate the possibility of specific drug targeting of antiviral drugs to the reticuloendothelial cells by the oral route. Hexylcyanoacrylate nanoparticles were used as colloidal drug carriers for Azidothymidine (AZT). 14C-labelled AZT was bound to nanoparticles using bis

(2-ethylhexyl) sulfosuccinate sodium as surfactant. The radioactivity in several organs including those containing large numbers of macrophages was measured after peroral administration of the nanoparticle preparation and compared to a [14C] AZT control solution containing the same components without the nanoparticles. In the liver the area under the curve (AUC) of [14C] AZT was 30% higher when the drug was bound to nanoparticles than after administration of the solution. In addition, higher [14C] AZT concentrations were observed after 1 hour and at later time points in blood and brain when nanoparticles were used compared to the control solution. These results indicate that nanoparticles are a promising drug targeting system for nucleoside analogues. Furthermore, the increase in drug availability at sites containing abundant macrophages, e.g. in the blood and in the brain, may allow a reduction in dosage and a decrease in systemic toxicity.

Kreuter *et al.,* (1997) Methylmethacrylate (MMA) sulfopropyl-methacrylate (SPM) copolymer nanoparticles were prepared by free radical polymerization and the loading characteristics of the muscarinic agonists arecaidine propargyl ester (APE) and pilocarpine were investigated. The loading efficiency was mainly influenced by the concentration of the copolymer carrier system and followed Langmuir's adsorption equation. The in vitro drug release was mainly influenced by

the composition of the acceptor phase used and the nanoparticle content of the donor phase. The bound drug was released from the carrier by competitive replacement by other ions from the binding sites of the particles. By this mechanism the nanoparticle system achieved a prolonged drug release, which was not the result of the increased viscosity at higher nanoparticle concentrations.

Kreuter *et al.,* (1997) Copolymer nanoparticles were investigated as carrier systems for the topical ophthalmic application of the muscarinic agonists arecaidine propargyl ester (APE) and (S)-(+)-aceclidine in rabbits and compared to conventional eye drop preparations. The copolymer nanoparticles were prepared by free radical polymerization of methylmethacrylate (MMA) and sulfopropylmethacrylate (SPM). The in-vivo activity of the drug-containing carrier systems was tested by the measurement of the miotic effect observed after local administration in rabbits. It has been found that the copolymer nanoparticles were able to produce a significant increase of the ocular APE bioavailability as determined by the area under the miosis-time-curve (AUC). The nanoparticle preparations were tolerated without any irritating effect in the rabbit eye. Besides the copolymer nanoparticles, different formulations containing bioadhesive or viscosity-enhancing polymers with and without additional nanoparticles were tested. The administration of APE-loaded

copolymer nanoparticles was found to be equivalent in efficacy to solutions containing the soluble polymers without nanoparticles. The combination of the nanoparticles with bioadhesive polymers further increased the ocular drug bioavailability. Hyaluronic acid alone or in combination with copolymer nanoparticles was observed to be the most effective soluble polymer for ophthalmic application by enhancing the AUC of miosis-time-curve 2-fold. Similar effects induced by the carrier systems were obtained with (S)-(+)-aceclidine. However, the magnitude of the enhancement of the miotic effect that is achievable by the binding of the drug to nanoparticles over free drug is much more pronounced with the short acting drug APE than with (S)-(+)-aceclidine.

Hagen Von Briesen *et al.,* (1996). Polyhexylcyanoacrylate nanoparticles loaded with either the human immunodeficiency virus (HIV) protease inhibitor Saquinavir (Ro 31-8959) or the nucleoside analog Zalcitabine (2,3-dideoxycytidine) were prepared by emulsion polymerization and tested for antiviral activity in primary human monocytes/macrophages in vitro. Both nanoparticulate formulations led to a dose-dependent reduction of HIV type 1 antigen production. While nanoparticle-bound Zalcitabine showed no superiority to an aqueous solution of the drug, a significantly higher efficacy was observed with Saquinavir-loaded nanoparticles. In acutely infected cells, an aqueous

solution of Saquinavir showed little antiviral activity at concentrations below 10 nM, whereas the nanoparticulate formulation exhibited a good antiviral effect at a concentration of 1 nM and a still-significant antigen reduction at 0.1 nM (50% inhibitory concentrations 5 4.23 nM for the free drug and 0.39 nM for the nanoparticle-bound drug). At a concentration of 100 nM, Saquinavir was completely inactive inchronically HIV-infected macrophages, but when bound to nanoparticles it caused a 35% decrease in antigen production. Using nanoparticles as a drug carrier system could improve the delivery of antiviral agents to the mononuclear phagocyte system in vivo, overcoming Pharmacokinetic problems and enhancing the activities of drugs for the treatment of HIV infection and AIDS.

Jean-Christophe Leroux *et al.,* (1995). CGP 57813 is a Peptidomimetic inhibitor of HIV-1 inhibitor protease. This lipophilic compound was successfully entrapped into poly (D, L- Lactic acid) (PLA) and pH sensitive methacrylic acid copolymers nanoparticles. The intravenous administration to mice of PLA nanoparticles resulted in a 2-fold increase of the area under the plasma concentration-time curve compared to a control solution. In vitro, these nanoparticles dissolved completely within 5 min at pH 5.8. PLA NPs which is insoluble in GIT.

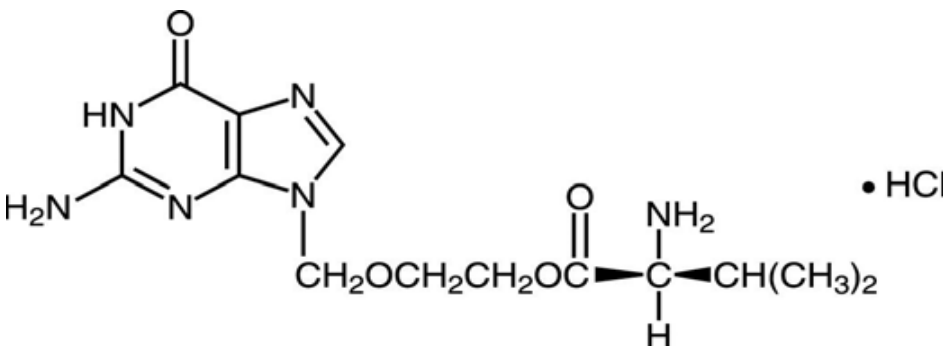
DRUG PROFILE

VALACYCLOVIR HYDROCHLORIDE

Hydrochloride salt of *L*-Valyl ester of the antiviral drug Acyclovir

Chemical Name : Valacyclovir hydrochloride is *L*-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl) methoxy] ethyl ester, monohydrochloride.

Structure :



Molecular Formula : $C_{13}H_{20}N_6O_4 \cdot HCl$

Molecular Weight : 360.80

Description and Solubility:

Valacyclovir hydrochloride is a white to off-white powder and maximum solubility in water at 25°C is 174 mg/mL.

MECHANISM OF ANTIVIRAL ACTION

Valacyclovir hydrochloride is rapidly converted to acyclovir which has demonstrated antiviral activity against herpes simplex virus types HSV-1 and HSV-2 and Varicella-zoster virus (VZV) both *in vitro* and *in vivo*. The inhibitory activity of Acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts Acyclovir into Acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. *In vitro*, Acyclovir triphosphate stops replication of herpes viral DNA.

This is accomplished in 3 ways:

1) Competitive inhibition of viral DNA polymerase,
2) Incorporation and termination of the growing viral DNA chain,
3) Inactivation of the viral DNA polymerase. The greater antiviral activity of Acyclovir against HSV compared with VZV is due to its more efficient phosphorylation by the viral TK.

CLINICAL PHARMACOLOGY

After oral administration, Valacyclovir hydrochloride is rapidly absorbed from the gastrointestinal tract and nearly completely converted to Acyclovir and L-valine by first-pass intestinal and/or hepatic metabolism.

PHARMACOKINETICS

Pharmacokinetics of Valacyclovir and Acyclovir after oral administration.

ABSORPTION AND BIOAVAILABILITY

After oral administration, Valacyclovir hydrochloride is rapidly absorbed from the gastrointestinal tract. The absolute bioavailability of Acyclovir after administration is 54.5% ± 9.1% as determined following a 1-gram oral dose of Valacyclovir hydrochloride. Acyclovir bioavailability from the administration of Valacyclovir hydrochloride is not altered by administration with food (30 minutes after an 873 Kcal breakfast, which included 51 grams of fat). There is no accumulation of Acyclovir after the administration of Valacyclovir at the recommended dosage regimens in healthy volunteers with normal renal function.

DISTRIBUTION

The binding of Valacyclovir to human plasma proteins ranged from 13.5% to 17.9%.

PROTEIN BINDING

Valacyclovir — Low (13 to 18%)

Acyclovir — Low (9 to 33%).

METABOLISM

After oral administration, Valacyclovir hydrochloride is rapidly absorbed from the gastrointestinal tract. Valacyclovir is converted to

Acyclovir and *L*-valine by first-pass intestinal and/or hepatic metabolism. Acyclovir is converted to a small extent to inactive metabolites by Aldehydeoxidase and by alcohol and Aldehyde dehydrogenase. Neither Valacyclovir nor Acyclovir is metabolized by cytochrome P450 enzymes.

HALF-LIFE

Valacyclovir: Less than 30 minutes

Acyclovir: After administration of Valacyclovir

Normal renal function—2.5 to 3.3 hours

End-stage renal disease—approximately 14 hours

Geriatric patients (65 to 83 years of age)—3.3 to 3.7 hours

Time to peak concentration

1.6 to 2.1 hours

PEAK PLASMA CONCENTRATIONS

Valacyclovir:

Plasma concentrations of unconverted valacyclovir are low, with peak concentrations of less than 0.5 mcg per mL (mcg/mL) after any dose. Plasma concentrations are nonquantifiable within 3 hours after administration.

Acyclovir:

Peak plasma concentrations are not proportional to the dose.

After a single dose of Valacyclovir.

500 mg : Approximately 3.3 mcg/mL

1 gram : 4.8 to 5.6 mcg/mL

After multiple doses of Valacyclovir

500 mg : Approximately 3.7 mcg/L

1 gram : 5 to 5.5 mcg/mL

ELIMINATION

Valacyclovir

Less than 1% of Valacyclovir is recovered unchanged in the urine over 24 hours.

In dialysis

It is not known if peritoneal dialysis removes Valacyclovir from the lood.

Acyclovir

Renal; Acyclovir accounts for 80 to 89% of the total urinary recovery. There was no accumulation of Acyclovir after repeated administration of Valacyclovir in patients with normal renal function.

In dialysis

Hemodialysis—during 4-hour hemodialysis session, approximately one third of Acyclovir in the body is removed. The half-life of Acyclovir is approximately 4 hours during hemodialysis.

Peritoneal dialysis

Chronic ambulatory peritoneal dialysis (CAPD) and continuous arteriovenous hemo filtration/dialysis (CAVHD) do not substantially remove Acyclovir, with pharmacokinetic parameters resembling those observed in patients with end-stage renal disease not receiving hemodialysis.

ADVERSE EFFECTS

Nausea

Headache

Vomiting

Dizziness

Abdominal pain

INCIDENCE LESS FREQUENT

Dysmenorrhea: Painful menstruation, including (abdominal cramps; diarrhea; nausea)

INCIDENCE RARE

Aplastic anemia

Chest pain; chills; cough; fever; headache; shortness of breath

Decreased consciousness

(Reduced mental alertness)— in patients with renal insufficiency

Hepatitis with liver function test abnormalities

Flu-like symptoms; unusual tiredness; yellow eyes or skin.

Renal insufficiency

Lower back/side pain; decreased frequency/amount of urine — manifested by increased serum creatinine.

Thrombocytopenia:

Black, tarry stools; chest pain; chills; cough; fever

Incidence not determined

Observed during clinical practice; estimates of frequency cannot be determined.

Acute hypersensitivity reactions and Anaphylaxis

Fast heartbeat; swelling of face; wheezing; difficulty in breathing or swallowing; skin rash; itching.

Aggressive behavior

Changes in behavior, especially in interactions with other people

Facial edema

Swelling or puffiness of face

Hallucinations

Seeing, hearing, or feeling things that are not there

Hemolytic anemia, Microangiopathic

Back, leg, or stomach pains; chills; difficulty breathing; swelling of face, hands, legs, or feet.

Hypertension

High blood pressure

Skin reactions such as erythema multiforme, photosensitivity, or rash:

Redness of the skin

Tachycardia

Fast, pounding, or irregular heartbeat; lightheadedness when getting up from a lying or sitting position

Strength(s) usually available

500 mg [*Valtrex*]

1 gram [*Valtrex*]

Packaging and storage

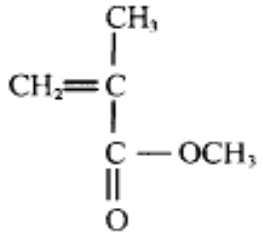
Store between 15 and 25 °C (59 and 77 °F), in a tight container. Protect from light.

MONOMER PROFILE

METHYL METHACRYLATE

Synonyms : 2-methacrylic acid methyl ester, 2-Propenoic acid, Methyl 2- methylpropenoate.

Structure



Methyl methacrylate is an organic compound with the formula $CH_2=C(CH_3)CO_2CH_3$. This colourless liquid, the methyl ester of methacrylic acid (MAA) is the monomer for the production of the transparent plastic polymethyl methacrylate (PMMA).

IUPAC Name	:	methyl 2-methlprop-2-enote
Molecular formula	:	$C_5H_8O_2$
Appearance	:	Colourless liquid
Molar mass	:	100.12 g/mol
Density	:	0.94 g/cm^3
Melting point	:	-48^{0}C (225 K)
Boiling point	:	101^{0}C (374 K)
Solubility in water	:	1.5g/100 ml (25^{0}C)

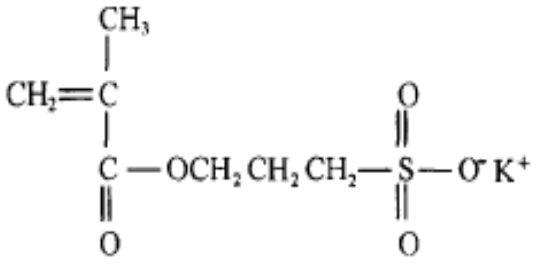
Viscosity : 0.6 cp at 20^0C

Category : Used for preparation of Polymethyl methacrylate.

: Used for production of co-polymers.

3-SULFOPROYLE METHACRYLATE, POTASSIUM SALT

Structure



Water soluble monomer. Used to introduce polar into polymer chains, confer shear stability to aqueous polymer dispersions.

Mol. Formula : $C_7H_{11}KO_5S$

Mol. Wt. : 246.3

Melting Point : 295^0C

Appearance : White powder

Solubility : Freely soluble in water

Stability : Stable, incompatible with peroxides, strong acids, strong oxidizing agents.

Storage : Store at 2-8^0C temperature. Protect from the

Sunlight

Category : Used for production of Co-polymers.

PREFORMULATION STUDIES

IDENTIFICATION TEST

Comparing of infrared spectrum of pure sample with the reference spectrum of Valacyclovir hydrochloride.

EQUIPMENT

1. FT- IR- Hydraulic pellet press.
2. Shimadzu, 8400 S.

PROCEDURE

The pure sample obtained is thoroughly mixed with completely dried potassium bromide and the pellet was prepared and the nature of the drug was analyzed by means of Shimadzu FT/IR.

COMPATIBILITY STUDIES

Infrared spectra matching approach was used for detection of any possible chemical interaction between the drug and polymer. A physical mixture (1:1) of the drug and polymer was prepared and mixed with suitable quantity of potassium bromide. About 100mg of the mixture was compressed to form a transparent pellet using a hydraulic press at 6 tons pressure. It was scanned from 4000 to 400 cm^{-1} in Shimazu FT/IR spectrophotometer. The IR spectrum of the physical mixture was compared with those of pure and polymers and matching was done to detect any appearance or disappearance of peaks.

Fig. 6. IR SPECTRUM OF VALACYCLOVIR . HCl

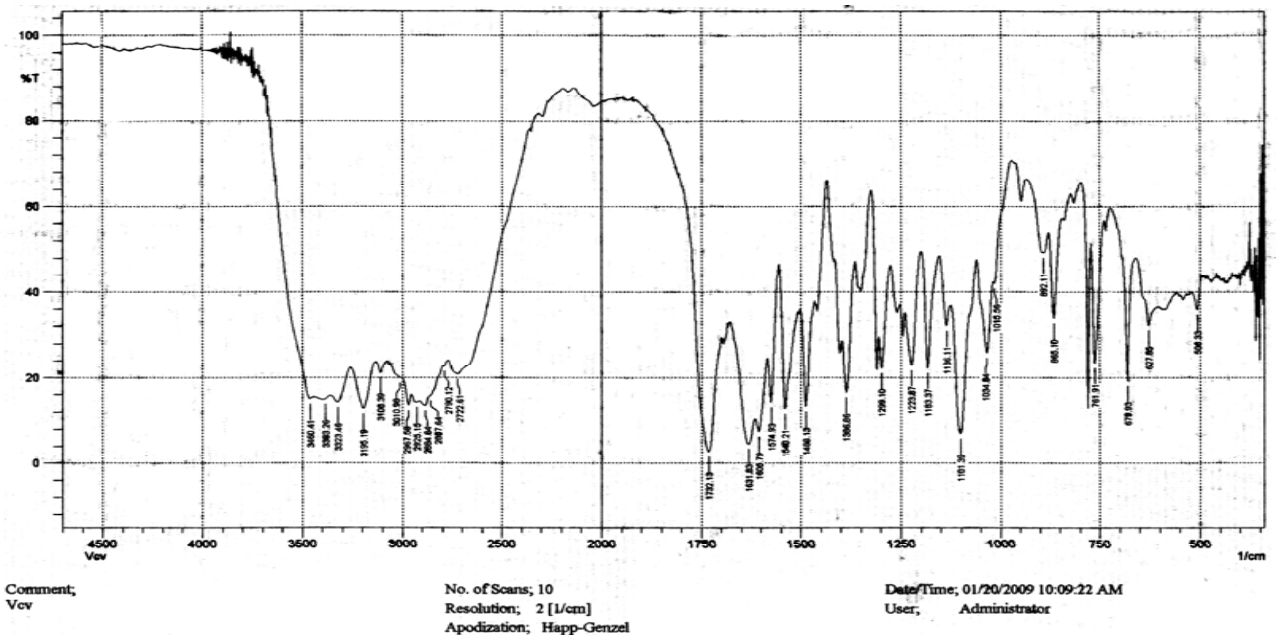


Fig. 7. IR SPECTRUM OF POLYMER (MMA-SPM)

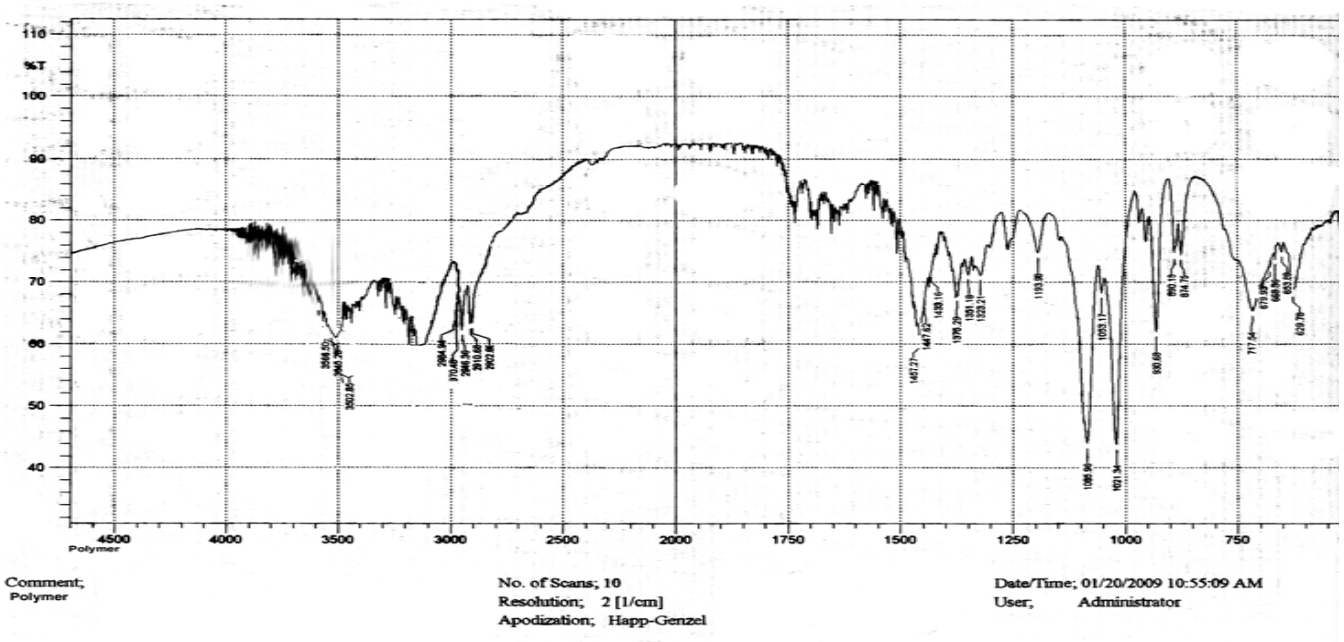
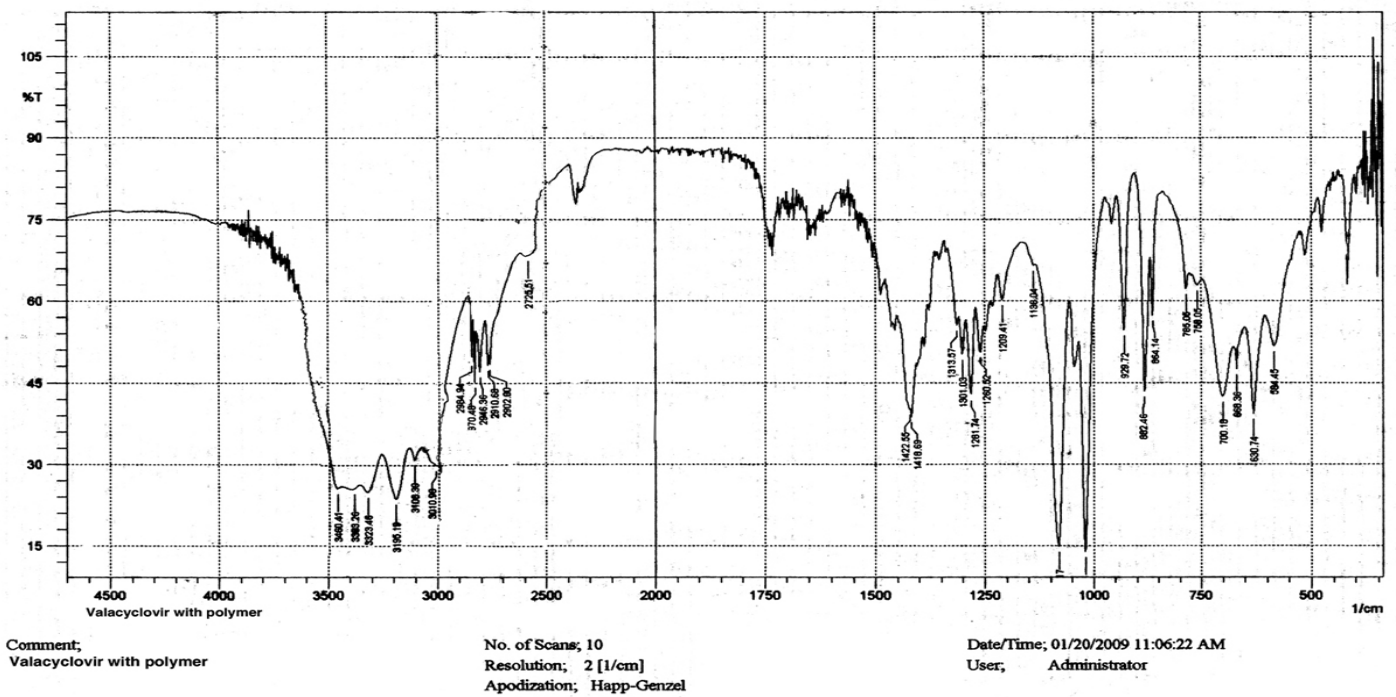


Fig. 8. IR SPECTRUM OF VALACYCLOVIR. HCl WITH MMA - SPM



Chapter VI
Preformulation Studies

FT-IR SPECTRAL ANALYSIS

Result

The compatibility between the drug and the selected polymers was evaluated using FT-IR peak matching method. The IR spectra of pure drug, polymers and the physical mixtures are shown in spectra. There was no appearance or disappearance of peaks in polymer and drug mixture, which confirmed the absence of any chemical interaction between drug and polymers.

EXPERIMENTAL WORK

MATERIALS & EQUIPMENTS USED FOR PREPARATION AND EVALUATION OF NANOPARTICLES

Materials Used

Materials	Source
Valacyclovir.Hcl	Aurobindo Pharmaceuticals, Hyderabad.
Methyl Methacrylate	Loba Chem. Pvt. Ltd, Mumbai.
Potassium dihydrogen Orthophosphate, Purified	HiMedia Lab. Pvt. Ltd, Mumbai.
3- Sulfopropylmethacrylate Potassium Salt	Sigma-Aldrich, USA.
Ammonium Persulphate	Fine Chem. Industry, Chennai.
D-Mannitol	HiMedia Laboratories Pvt. Ltd. Mumbai.
Acetonitrile	Ranbaxy Fine Chemicals Ltd, Mumbai.
Methanol	Loba Chem. Pvt. Ltd, Mumbai.
Sodium hydroxide	S.D Fine Chem. Ltd., Mumbai.
Potassium dihydrogen orthophosphate	Loba Chem. Pvt. Ltd, Mumbai.
Dulbecco's Phosphate buffered saline (DPBS)	HiMedia Laboratories Pvt. Ltd. Mumbai.
Polysorbate-80	Loba Chem. Pvt. Ltd, Mumbai.
Dialysis membrane	HiMedia Laboratories Pvt. Ltd. Mumbai.
Dialysis Closure Clips	HiMedia Laboratories Pvt. Ltd. Mumbai.
HPLC grade water	Ranbaxy Fine Chemicals Ltd, New Delhi.
Deionised water	PSG College of Pharmacy, Coimbatore.

Chapter VII
Experimental Work

EQUIPMENTS USED

Equipments	Model/Company
Digital Balance	Shimadzu Electronic balance
Magnetic stirrer	Remi Electronics
Freeze drier	Labconco
Cooling Centrifuge	Eppendroff Centrifuge
Vacuum filter	Merck Ltd
FT-IR spectrometer	Shimadzu, 8400 S Model, 2006.
HPLC	Shimadzu, LC- 10 ATUP, 2005.
Scanning Electron Microscopy	JEOL, Model no JSM 6360

Chapter VII
Experimental Work

STANDARD GRAPH OF VALACYCLOVIR .HCL

Procedure:

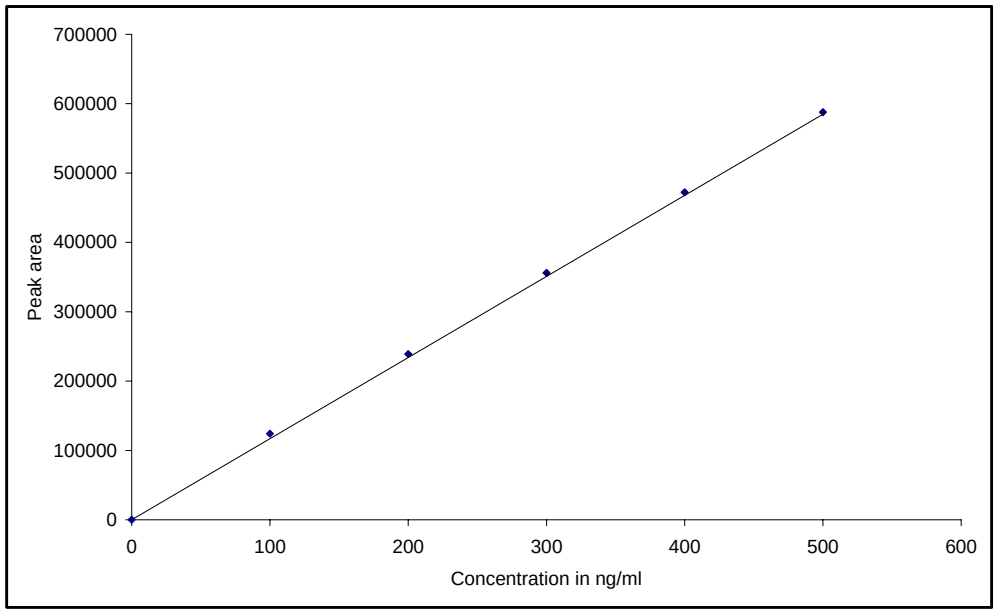
A Stock solution of Valacyclovir hydrochloride was prepared by dissolving 10 mg of pure drug in 10 ml of mobile phase at pH 6.5 to give 1000 µg/ml. Then 1ml of stock solution was diluted to 10ml using mobile phase to produce 100µg/ml. From this working solution, dilutions were made to produce 100, 200, 300, 400, and 500 ng/ml.

The UV detector was set at a wavelength of 271nm in RP-HPLC. An injection volume of 20 µL was used.

Table .1 Standard graph of Valacyclovir. HCl

S. No	Concentration ng/ ml	Peak area
1.	0	0
2.	100	131871
3.	200	244829
4.	300	365497
5.	400	472283
6.	500	587952

Fig. 9 Standard graph of Valacyclovir. HCl



Chapter VII
Experimental Work

PREPARATION OF BUFFER SOLUTIONS

Preparation of Dulbecco's phosphate buffered saline

Dulbecco's phosphate buffered saline – (Bisodium phosphate/monobasic Potassium phosphate/Sodium chloride/ Potassium chloride (w/w/w/w = 1.15:2:8:0.2). Place 0.96 gm of DPBS in 100 ml standard flask add distilled water to dissolve and make up the volume.

Preparation of 3N NaOH solution

Place 12gm of sodium hydroxide pellets in 100 ml standard flask add distilled water to dissolve and make up the volume.

Preparation of 0.067 M KH_2PO_4 solution

Place 0.813 gm of potassium dihydrogen phosphate (KH_2PO_4) in 100 ml standard flask add distilled water to dissolve and make up the volume.

Preparation of mobile phase for standard graph in HPLC

The mobile phase prepared by mixing the Acetonitrile: Methanol: 0.067 M KH_2PO_4 (27:20:53, V/V/V) in 1000 ml standard flask and adjusted to pH 6.5 with 3M sodium hydroxide and delivered at the flow rate of 1Ml/min. (Ayhan Savaer et al., 2003).

Chapter VII
Experimental Work

Preparation of 0.2 m potassium dihydrogen phosphate buffer

Place 62.5ml of 0.2M potassium dihydrogen phosphate in a 250ml volumetric flask, add the specified volume of 0.2M Sodium hydroxide and then add water to 250ml volumetric flask.

Preparation of 0.2M potassium dihydrogen phosphate

Place 27.218gm of Potassium dihydrogen Phosphate pellets in 1000 ml standard flask add distilled water to dissolve and make up the volume.

Preparation of 0.2M sodium hydroxide

Place 8.4gm of Sodium hydroxide in 1000ml standard flask add distilled water to dissolve and make up the volume.

Chapter VII
Experimental Work

FORMULATION OF VALACYCLOVIR MMA-SPM NANOPARTICLES

Preparation of Valacyclovir loaded Nanoparticles

		For 20ml
Valacyclovir	-	20mg
Ammonium persulfate 0.03% (w/v)	-	6mg
Methyl methacrylate 4.95% (w/v)	-	0.99 ml
Sulfopropyl methacrylate potassium Salt 0.05% (w/v)	-	10mg
D- Mannitol 4% (w/v)	-	240mg
Dulbecco's phosphate buffer saline	-	q. s
Polysorbate-80 0.01% (w/v)	-	2mg

Valacyclovir Nanoparticles formulated by free radical polymerization method. In this method 10mg of sulfopropyl methacrylate (SPM) was mixed with 0.99ml of methyl methacrylate (MMA) in ultrapure deionized water. 6mg of Ammonium persulfate was added as an initiator, into the above solution under constant magnetic stirring at 78^{o}C in 400 rpm over a period of 24 hrs to form methyl methacrylate sufopropyl methacrylate nanoparticle. MMA-SPM NP suspension was filtered through a filter paper pore size of 0.8 µm and lyophilized with 4% mannitol. The 20 mg of drug was added with pH adjusted with Dulbecco's phosphate buffer saline and allows stirr for 150 rpm at 37^{o}C for 3 hrs. Then the 0.01% of polysorbate-80 was added to stabilize the formulation and it is lyophilized to form a powder of nanoparticles (Yung- Chin Kuo., (2005), F.Stieneker *et*

al., (1996), J. Kreuter *et al*., (1997).

MECHANISM OF FORMATION OF NANOPARTICLES

Free Radical Polymerization Method

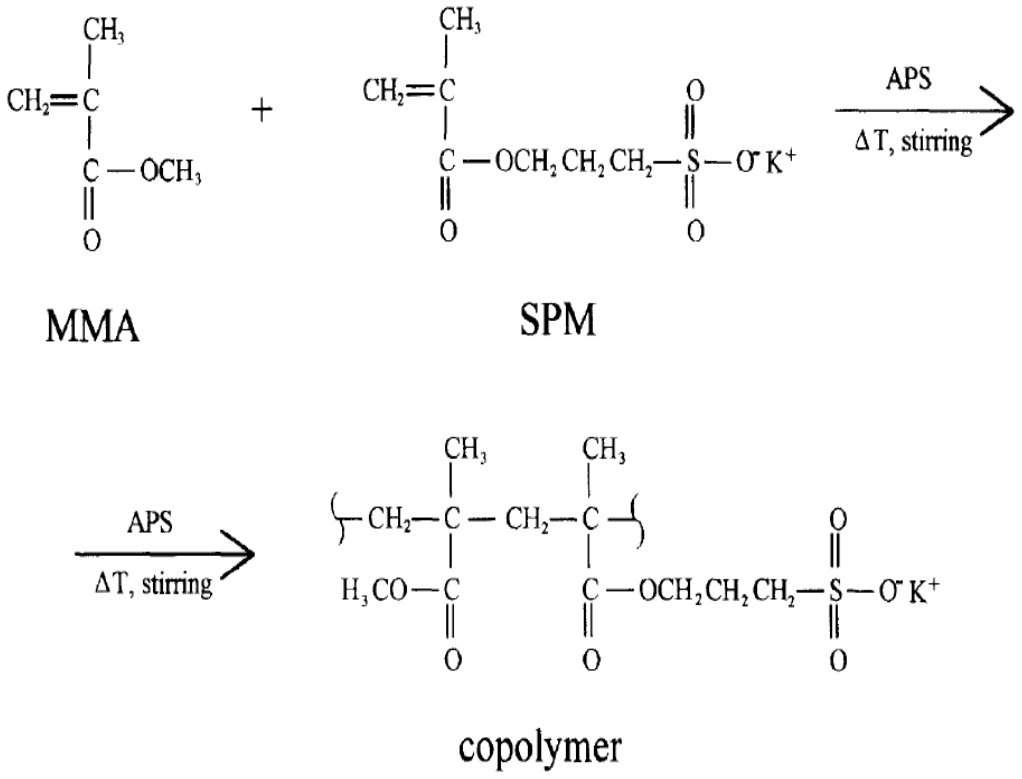


Fig. 10. Procedure for preparation of Valacyclovir Nanoparticle

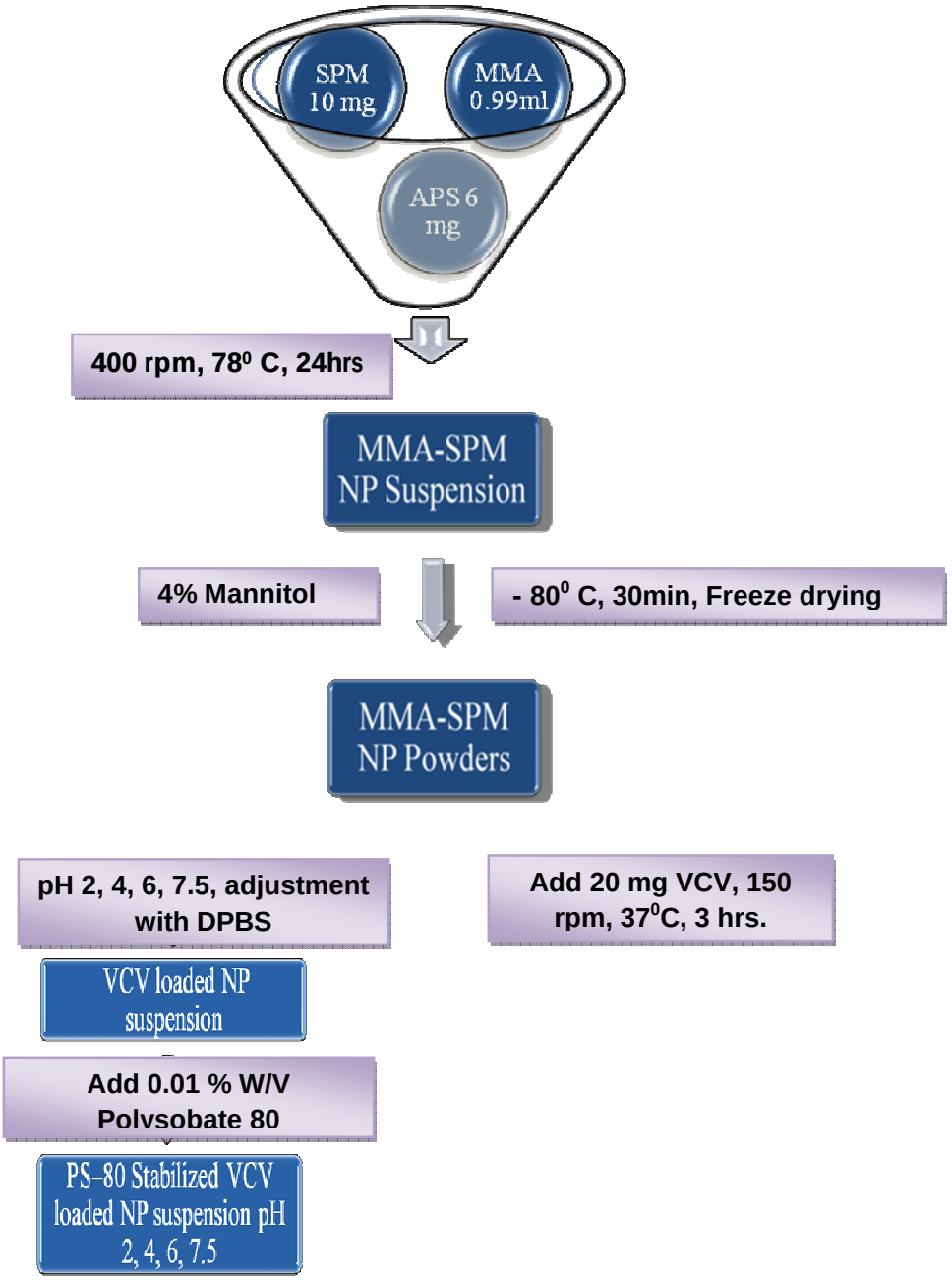


Table No. 2

Preparation of Valacyclovir MMA -SPM Nanoparticles

S. No	Formulation Code	Formulation pH	Drug Concentration in mg/ml	Volume of MMA in ml	Amount of SPM in mg
1.	NP_1	2	20	0.99	10
2.	NP_2	4	20	0.99	10
3.	NP_3	6	20	0.99	10
4.	NP_4	7.5	20	0.99	10

Chapter VIII
Evaluation of Nanoparticles

IN VITRO EVALUATION OF VCV MMA-SPM NANOPARTICLES

METHODS

1. Scanning electron microscopy (SEM) analysis
2. Drug content and percentage drug loading
3. Encapsulation efficiency
4. *In vitro* release studies

1. Scanning electron microscopy (SEM) analysis

The morphology of Valacyclovir MMA-SPM nanoparticles was examined by Scanning electron microscopy (SEM) JEOL JSM- 6360. The solid sample for SEM analysis was coated with a thin layer of platinum using the physical vapor deposition (PVD) process at 30mA current from the distance of 50 mm during 180 seconds.

2. Determination Drug content and percentage drug loading

To determine the drug content and percentage drug loading, 1.5 ml of Valacyclovir loaded MMA-SPM nanoparticle formulation (NP1, NP2, NP3 & NP4) was taken in eppondrof tube and centrifuged at 10,000 rpm at 4°C for 3 hrs and the supernatant was analyzed in RP-HPLC at 271 nm. (Yung- Chin Kuo., 2005).

$$Drug\ \ Loading = \frac{Total\ Weight\ of\ \ Drug - Drug\ in\ Supernatant}{Total\ Weight\ of\ \ Drug}\ X\ 100$$

3. Determination of encapsulation efficiency

The encapsulation efficiency of formulation was determined by the followed equations. Encapsulation efficiency (E.E) was defined as the percentage of determined loading relative to the nominal (theoretical) loading (Yung-Chin Kuo et al 2005).

$$E.E = \frac{Total\ volume - The\ Amount\ of\ Drug\ in\ total\ volume}{Total\ volume} X 100$$

4. *In vitro* drug release studies

In vitro release of Valacyclovir from formulations was measured by using a dialysis membrane 110 (12,000 da, HiMedia Lab. Pvt. Ltd, Mumbai). MMA-SPM nanoparticles were centrifuged in 10,000 rpm at 40 C for 3 hrs. The supernant was decanted the sediment suspension was preserved for *in vitro* release studies. 2 ml of the preserved suspension was filled in dialysis membrane and the packed membrane was immersed in 50 ml of Phosphate buffered saline (PBS) of pH 7.4 which was used as a medium at 37± 10C under constant stirring. At preset time intervals, 2ml of aliquots of the medium were withdrawn and the same volume was replaced by fresh medium to maintain the sink condition. The sample was analyzed by RP- HPLC. (Masayuki yokoyama et al., (2005).

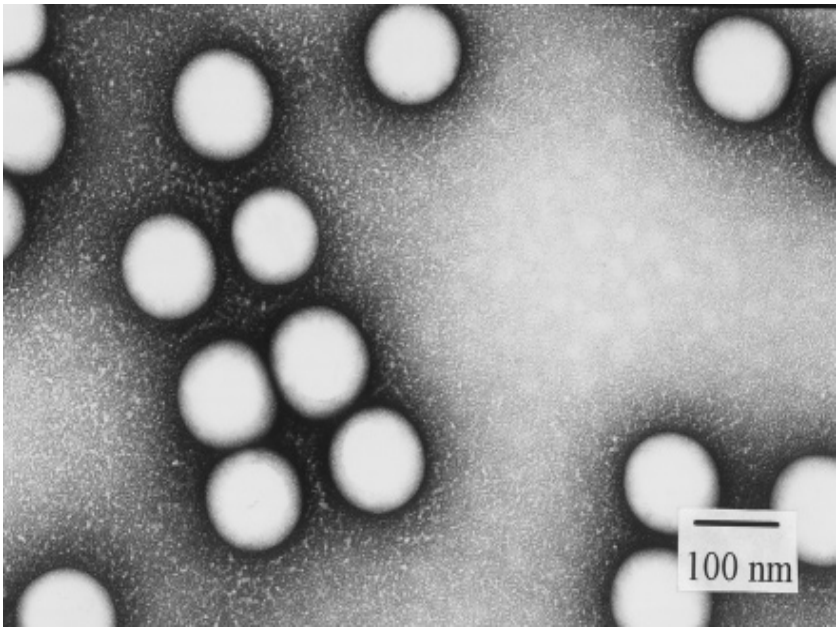
Percentage of drug released (%) = (a/b) x 100

a = Amount of VCV released, b = Amount of VCV entrapment

RESULTS AND DISCUSSION

SEM ANALYSIS

Fig.11 SEM picture of Valacyclovir loaded MMA-SPM Nanoparticles



DRUG CONTENT AND PERCENTAGE OF DRUG LOADING

Table : 3 Drug content and drug loading efficiency of VCV MMA-SPM NP

Formulation Code	pH of VCV MMA-SPM NP formulation	Peak area	Concentration in ng/ml	Drug Content in 1.5 ml of VCV MMA-SPM NP formulation (ng)	Percentage of drug loading efficiency (%)
NP1	pH 2	58265	88.2	1.42	71
NP2	pH 4	49532	75.1	1.51	75.5
NP3	pH 6	43443	65.9	1.57	78.5
NP4	pH 7.5	40911	62.1	1.59	79.5

Fig. 12 Drug loading chromatogram of RP-HPLC VCV MMA-SPM NP

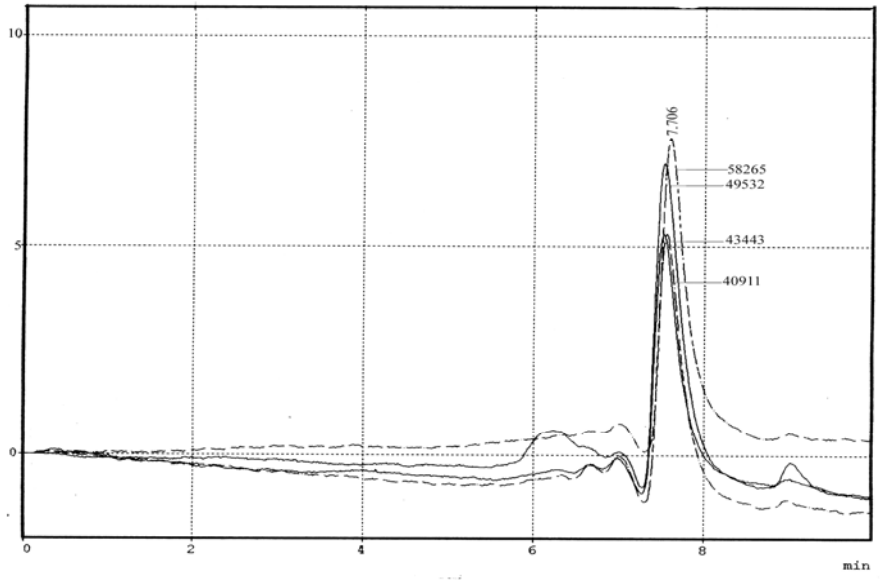

10
5
0
0
2
4
6
8
min
7.706
58265
49532
43443
40911

Fig. 13. Drug content analysis

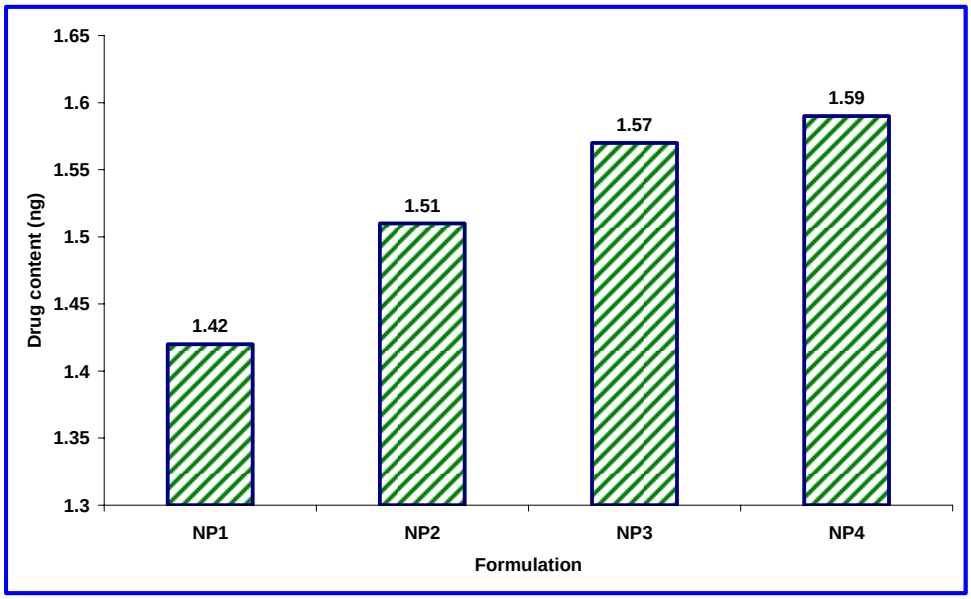
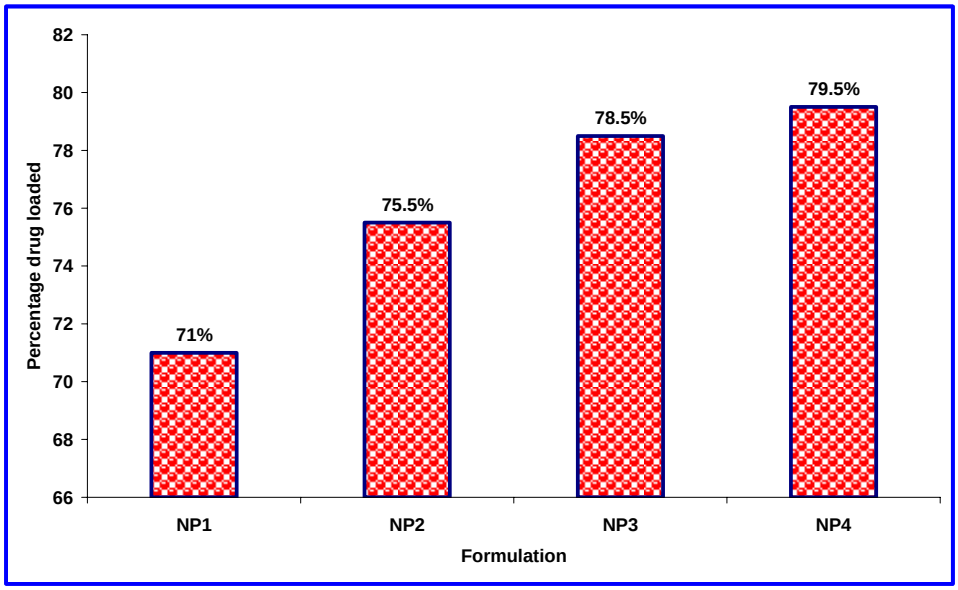


Fig. 14. Percentage of drug loading efficiency



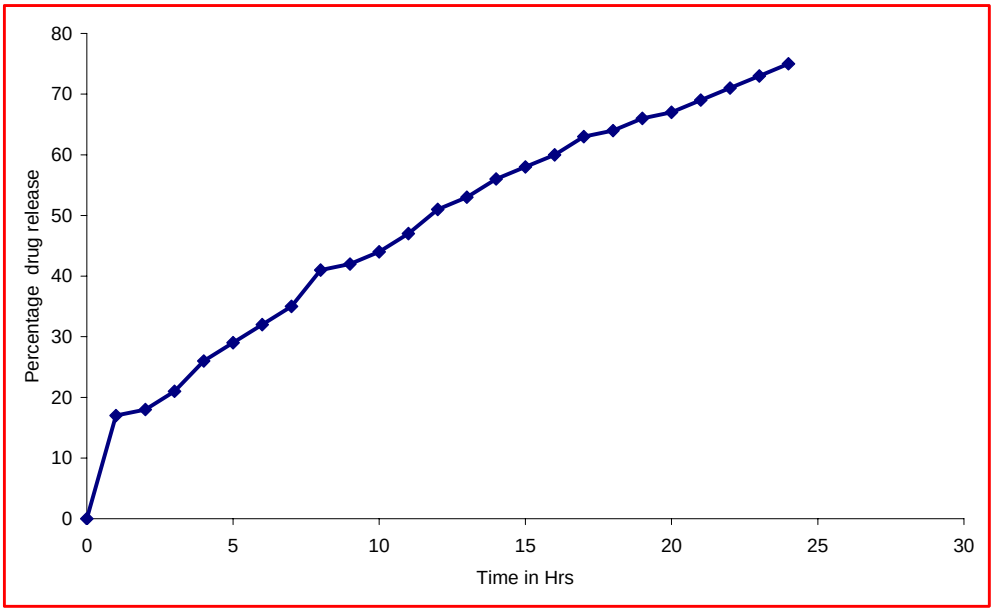
IN VITRO RELEASE STUDIES

Table: 4 *In vitro* drug release profile of Valacyclovir Nanoparticles (NP4) by RP-HPLC

Time in Hrs	Peak area	Percentage drug release
1	98046±60.34	17±0.91
2	102280±99.17	18±0.86
3	120255±78.57	21±0.72
4	144512±97.62	26±0.78
5	165743±88.56	29±0.74
6	182716±82.32.	32±0.58
7	198974±64.32	35±0.59
8	228918±75.61	41±0.43
9	234876±90.52	42±0.41
10	249452±102.3	44±0.39
11	264029±96.68	47±0.35
12	287254±85.26	51±0.32
13	297143±88.01	53±0.30
14	312784±42.56	56±0.28
15	326248±77.80	58±0.26
16	335931±34.48	60±0.24
17	349783±49.54	63±0.27
18	356551±59.41	64±0.23
19	368498±67.67	66±0.20
20	376629±96.93	67±1.20
21	383248±99.01	69±1.43
22	396522±84.12	71±1.46

23	404893±87.65	73±1.47
24	417821±79.43	75±1.49

Fig. 15 *In vitro* drug release profile of Valacyclovir Nanoparticles at different time intervals



1. Particle size and surface analysis using SEM

Scanning electron microscopy reveals that all prepared nanoparticles had a homogeneous solid matrix structure, with no evidence of crystals on the surface (Fig.11). The Valacyclovir loaded nanoparticles prepared by free radical polymerization method enables us to get spherical, discrete spheres with a size ranging from 80 t0 110nm.

2. Drug content and percentage drug loading

The results of drug content and percentage drug loading was shown in table. The results were suggesting that the nanoparticles loaded in pH 7.5 was found to have high drug content while compared to other pH conditions. The loading efficiency is found to be pH dependent which is directly proportional. The drug content of formulations NP1, NP2, NP3 and NP4 were found to be 1.42, 1.51, 1.57 and 1.59 respectively. The percentage of drug loading was found to be 71%, 75.5%, 78.5% and 79.5%.

3. Percentage Encapsulation efficiency

The results of encapsulation efficiency was shown in table. The encapsulation efficiency of formulations NP1, NP2, NP3 and NP4 was

found to be 29%, 24.5%, 21.5% and 20.5% respectively. It is evident that the pH is indirectly proportional to the percentage encapsulation efficiency.

4. *In vitro* release studies

The MMA-SPM nanoparticles loaded with Valacyclovir in pH 7.4 (formulation NP4) was found to have high drug content and high percentage loading which found to be the best formulation compared to other formulation. So, NP4 was selected for the in vitro release studies. The in vitro drug release profile was shown in table & fig. the in vitro release study was carried out in pH 7.4. The results suggest that the release was found to be sustained for more than 24 hrs. Around 75% of drug was released at the end of 24 hrs. the outcome of release profile indicating that the MMA-SPM nanoparticles loaded with Valacyclovir can be used as controlled release formulation.

SUMMARY AND CONCLUSION

Herpes simplex virus (HSV) is a human DNA virus with two species, HSV-1 and HSV2, that causes a variety of disease manifestations. The major public health importance of HSV2 lies in its potential role as a cofactor for HIV transmission. Valacyclovir is an oral antiviral drug which included in the antiretroviral therapy prolongs survival in HIV seropositive individuals. The Valacyclovir is having low bioavailability profile with toxicity and adverse reactions in high dose.

The present work was proposed to prepare MMA-SPM nanoparticles loaded with Valacyclovir to achieve better bioavailability with lowest possible dose. From the plethora of literatures it was found that MMA-SPM NPs are suitable carrier for hydrophilic or even charged molecules (Langer *et al.,* 1997). The MMA-SPM nanoparticles were prepared and the drug was loaded in different pH environment.

The prepared NPs are evaluated for different parameter. The particle size and surface morphology results revealed that the nanoparticles are in the size range of 80 – 110 nm. Which may be useful for targeting the Nps to macrophages or monocytes. The *in vitro* release

suggests that the Nanoparticles shows a better controlled release profile

for more than 24 hrs. The frequency of the doses can be reduced due to the controlled release nature. The NPs is targeted delivery devices which will be useful to reduce the dose level of Valacylclovir.

To conclude, the prepared nanoparticles may be used to achieve the better bioavailability profile with targeting to the macrophages. The *in vivo* studies may be carried out to optimize the therapeutic response of the prepared nanoparticles.

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